



Editor

Prof. Alaeddin BOBAT, Ph.D

**PIONEER AND
CONTEMPORARY STUDIES
IN AGRICULTURE, FOREST
AND WATER ISSUES**

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Chapter 1

Seawater Desalination

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ABSTRACT

Humankind has found life near water resources and struggled to own it in order to secure itself. However, the owned water resources have started to be consumed a lot with the increasing population over time, urbanization, industrialization and agricultural irrigation activities, and they have gradually decreased due to the effects of climate change. The inadequacies in water resources, the ever-increasing need for drinking and utility water have brought to the agenda the treatment of sea water to meet the water supply. Although the investment and operating costs of the plants that treat sea water are high, many countries provide fresh water production with this method. In recent years, there have been significant reductions in unit costs as a result of the use of renewable energies as an energy source with the technological studies carried out in this field. Looking at the distribution of seawater desalination technologies today, multistage flash distillation (MSFD) which is a thermal process and reverse osmosis (RO) plants, which is a membrane process, constitute more than 85% of the total facilities. Although these plants are approximately evenly distributed, the common trend is to prefer membrane processes. The advantage of the reverse osmosis method is that it takes up less space or small-scale facilities can be built and modularly. The amount of energy used in reverse osmosis facilities is half of that used in other facilities. Because, in reverse osmosis method, mechanical energy is used as opposed to the thermal energy used to heat and evaporate the water, as in the thermal methods. This results in lower operating and maintenance costs. In recent years, hybrid desalination systems combining thermal and membrane desalination processes are considered to be a good economical alternative. In this sense, hybrid membrane processes that combine multistage flash distillation (MSFD) and reverse osmosis (RO) processes come to the fore.

Keywords: Seawater, Desalination, Desalination technologies, Reverse osmosis, Avşa Island.

1. INTRODUCTION

World is the only astronomical object in the solar system that, as far as we know, has life and liquid water on it. According to radiometric dating and other evidence, it was formed more than 4.5 billion years ago.

About 29% of the Earth's surface is covered by land consisting of continents and islands, while the remaining 71% is covered by oceans, seas, lakes, streams and other fresh waters.

Oceans and seas make up 97.4%, polar ice caps 2.0%, underground waters 0.5%, and lakes and rivers, especially soil moisture, the remainder, of the total amount of water with a total asset of 1.4 billion km³. Every year, 70 thousand km³ of the 110 thousand km³ of precipitation falling on the land is returned by evaporation. 40 thousand km³ of water is the world's annual renewable water resource.

Although this amount of water is often enough for the living conditions on land, it is not evenly distributed on the earth, and from time to time, its scarcity or absence is experienced.

In this sense, humankind has found life near water resources and struggled to own it in order to secure itself. However, the owned water resources have started to be consumed a lot with the increasing population over time, urbanization, industrialization and agricultural irrigation activities, and they have gradually decreased due to the effects of climate change.

The inadequacies in water resources, the ever-increasing need for drinking and utility water have brought to the agenda the treatment of sea water to meet the water supply. Fresh water is produced by desalination sea water in order to meet the need for domestic water, including drinking water, in touristic facilities and sites on the sea coasts and in marine vessels.

Although the investment and operating costs of the plants that treat sea water are high, many countries provide fresh water production with this method. In recent years, there have been significant reductions in unit costs as a result of the use of renewable energies as an energy source with the technological studies carried out in this field.

In this section, it is aimed to compile technical and economic information about the methods of supplying drinking and utility water from sea water.

2. WORLD WATER RESOURCES

Water, the main source of life on earth, is a natural asset that cannot be replaced by any other source. Fire, soil and air which is the main element in the creation of the universe together with is one of the four beings called "Anasır-ı Erbaa". It is a limited resource that must be protected.

Nature is in dynamic balance in terms of the amount of water. Water is an inexhaustible natural resource and the total amount of water in the world does not change over time. Considering a long period of time, the amounts of water entering and leaving any part of the water cycle are equal. For example, the precipitation that falls on earth in a year is equal to the amount of water that evaporates and returns to the atmosphere in that year.

A very large part (97.4%) of the water distributed between various parts of the earth is in the seas (hydrosphere). The distribution of 36.12 million km³ of fresh water on land (lithosphere) and in the air (atmosphere) is as follows. 77.23% in polar ice, 12.35% in deep groundwater, 9.86% in near-surface groundwater, 0.35% in lakes, 0.17% in soil moisture, 0.04% in atmosphere, 0.003% in rivers and it is 0.003% in living beings. As can be seen, the percentage of water in the atmosphere, streams and living things at any given time is very low.

3. SEA WATERS

Ocean and sea waters, which make up 97.4% of the world's water supply, are not drinkable. The important properties of this source, which is outside the drinking and utility water quality standards, are temperature, salinity, pH, density, particulate matter and dissolved gases.

Earth is the only planet in the solar system that has seawater. The total volume of seawater is about $1,348 \times 10^6$ km³, and the annual evaporation is about 450×10^3 km³. Therefore, the time required for all ocean water to be lost due to evaporation is about 3,000 years. The amount of sea water is preserved, as the amount of evaporated water is compensated by precipitation, runoff from rivers, and ice wrecks from the northern and southern seas.

3.1. Origin of sea water

There are various theories about how the current seas were formed. This is closely related to how the world itself was formed and its history. Water, which is the source of life for all living things, according to one of the two general views, with the release of water vapour along with the gases erupting from the constantly erupting volcanoes, and this creates clouds and therefore rains; According to the other, it was formed as a result of small comets formed from glaciers and frozen asteroids colliding with the Earth.

In fact, these two general views can be much more meaningful when expressed as a single theory. Although it is not known how many celestial bodies hit the earth and how much water it left, it is estimated that this bombardment continued from 4.5 billion years ago to 3.8 billion years ago

when the world was formed._When this period is over, there are now oceans in the world._They appeared in living organisms about 1 billion years later.

3.2. Taking seawater samples

In order to determine the physical and chemical properties of sea water depending on depth and location, samples taken from sea water are analysed in the laboratory._Surface seawater can be easily removed from the ship using a bucket or pump._However, it is not always easy to take water samples from a depth of thousands of meters without mixing with other sea water and the water temperature is known._That is why the water sampler (Nansen water sampler), designed by the Norwegian oceanographer F. Nansen in 1904, is the most widely used in the world._This water sampler is lowered to the desired depth depending on the wire unloaded from the crane of the observation vessel._With a mercury thermometer in contact with sea water at a certain depth, approximately 1-2 litres of sea water sample can be taken, the temperature of which is also recorded._Most of the ocean observation data available so far has been obtained using this Nansen water sampler

In recent years, with the increase in research on the determination of seawater chemical properties, water sampling devices that can take much more samples have been designed._Plastic water sampling devices have been developed that can take large amounts of seawater samples at the same time.

3.3. Physical and chemical properties of sea water

Sea water has a fluid characteristic, which is a general characteristic of liquids._Therefore, sea water is in constant motion under the influence of thermal radiation from the sun and wind._Various physical properties of seawater add depth to this movement, as surface tension, for example, plays an important role in the formation of waves.

Some ions found in seawater exist as complex ions, while others exist in combination with water._Therefore, the molecular structure of sea water is different from that of pure water._When describing the physical properties of sea water, temperature and salinity are usually the first ones that come to mind. However, it is important density, dissolved gases, pH, particulate matter, and optics.

According to the analysis of sea water collected from the world's oceans, the amount of salt in sea water varies slightly depending on location and depth, however, the concentration ratio of the main elements was found to be constant.

Sea water contains most of the chemical elements found on earth._These are mostly in the form of salt solutions._There is an average of 34.9 grams of salt in

one kilogram of seawater. Accordingly, the amount of salt in the oceans and seas is calculated as 5×10^{16} tons. The amount of salts present varies from place to place. The salts found in the oceans and seas and their rates are given in Table 1, and the dissolved gases and their ratios are given in Table 2.

Table 1. Major chemical elements in seawater

Chemical elements	g / L	%
Chlorine	18,980	55,04
Sodium	10,556	30,61
Magnesium	1,272	3,69
Sulphates	2,649	7,68
Calcium	0,400	1,16
Potassium	0,380	1,10
Bicarbonates	0,140	0,41
Strontium	0,013	0,04

Table 2. Major gases in seawater and atmosphere

Gases	Atmosphere	sea water
Nitrogen	78,09	62,6
Oxygen	20,95	34,2
Argon	0,93	1,6
Carbon dioxide	0,03	1,5

As can be seen, nitrogen is present in the seas at a lower rate than in the atmosphere. However, it is still the most abundant gas. Surface waters are rich in this regard because they take oxygen from the atmosphere. As you go below the surface, the amount of oxygen decreases.

3.3.1. Heat

The seas have a high thermal capacity as they trap heat. The sun's rays reach depths of up to 100 m from the sea surface and most are absorbed at 10 m. For this reason, the water temperature at the sea surface and near the surface varies both seasonally and daily. The most important factors affecting sea water temperature are latitude, salinity, cold water currents and wind direction during the day. Generally it is between -2°C (freezing point) to 30°C depending on the season, sea area and depth.

Due to the very low thermal conductivity of water, the heat generated is carried to the depths of the sea by means of mixing. This mixture, which occurs with waves, winds and other factors, can go down to 200-300 m from the sea surface. The region between the surface of the water and the sea floor, where the temperature changes much faster than the other layers according to the

depth, is called the thermocline layer. Below this layer, the sea water temperature drops to 5 °C, and to 0-3 °C at deeper levels.

For example, the average sea water temperatures for many years have been 22.4 °C in the Mediterranean, 19.8 °C in the Aegean Sea, 17.4 °C in the Marmara Sea and 16.5°C in the Black Sea.

3.3.2. Salinity

Minerals dissolved from rocks on the Earth's surface are the source of the salinity of seawater. Every year, 2.3 billion m³ of minerals are transported to the oceans in this way. Volcanic volcanoes and gases from eruptions are also sources of large amounts of chlorine, sulphur, bromine and boron to this water. However, the amount of the ion Cl⁻, which has the greatest effect on salinity in sea waters, emerged with the dissolution of gaseous HCl in sea waters, which was caused by volcanic movements in the first periods of world history.

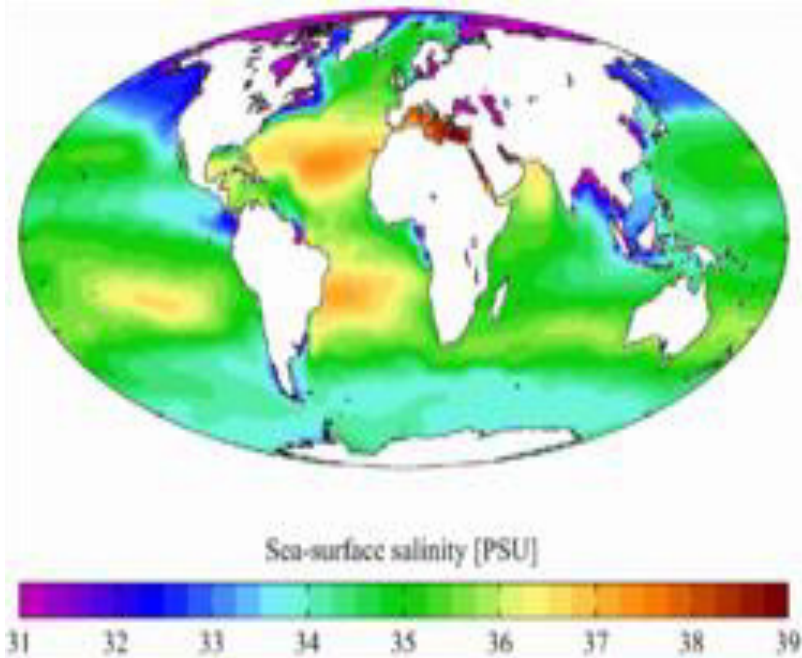
Although all known elements are found in sea water, the main ones causing salinity are chlorine (Cl⁻), sodium (Na⁺), magnesium (Mg⁺²), sulphate (SO₄⁻), calcium (Ca⁺²), potassium (K⁺²).), bicarbonate (HCO₃⁻), bromide (Br⁻), borate (H₂BO₃⁻), strontium (Sr⁺²) and fluoride (F⁻).

Salinity in the seas varies between 2.8 - 4.0% depending on the differences between evaporation and precipitation. The weighted average salinity is 3.5% (35 g of dissolved salt per kilogram or liter of seawater / the electrical conductivity value standardized to 25 °C, which is an indicator of the total ion content of seawater, is 35,000 μS/cm). The averages of seas inflowing from closed seas, estuaries and rivers with less salinity contain less salt than the normal average values. The saltiest open sea is the Red Sea. Excessive evaporation, low precipitation, scarcity of river water flowing into the sea and restricted water circulation are the main causes of this situation. In closed basin seas such as the Dead Sea, the salinity is considerably higher. The world annual average sea surface salinity map is given in Figure 1.

Despite this amount of salt being transferred, the salinity of the oceans remains constant without increasing continuously because it is used at the same rate and speed. These substances are used or deposited by marine organisms for shell and skeleton construction.

Salinity generally increases with increasing depth. The depth between 100 m and 1,000 m, where salinity changes rapidly, is called the halocline layer. Again, in the summer months, the increase in salinity occurs towards the surface due to intense evaporation.

Salinity can be measured by different methods. However, since it is more practical and reliable, the determination of salinity is made with electrical conductivity measuring instruments.



Source: World Ocean Atlas.

Figure 1. World annual average sea surface salinity map

3.3.3. pH

The normal pH of seawater is between 7.5 and 8.5. The factor affecting this value is CO_2 and carbonate ions. At these pH values, there is 88.9% HCO_3^- in water. HCO_3^- ions combine with H^+ ions and turn into H_2CO_3 , which is a buffer solution. H_2CO_3 is pacing the pH of the water to normal levels.

The natural colour of sea water is blue. Water absorbs illuminating rays at uneven rates. Transparent water appears blue because red rays are absorbed more than blue rays. Plankton and suspended solids can distort the natural colour of water, blue, and turn it into yellow-green. Sea water also contains suspended organic and inorganic substances of various structures and sizes.

As we go deeper in sea water, the dissolution of CO_2 resulting from biological respiration increases with decreasing water temperature and increasing pressure. Therefore, the pH value in deep waters is lower than the surface. However, this does not occur in shallow and warm waters.

3.3.4. Intensity

Density is an important factor affecting the stratification of seawater and the formation of currents. Since the density of wastewater, which is generally discharged from the bottom, is lower than sea water, it rises towards the surface and mixes with sea water and dilutes. Meanwhile, they rise to the surface or remain at a certain depth, depending on the existing stratification.

The density of seawater varies with pressure, salinity, temperature and depth. Sea water is denser than pure water, which has a density of $1,000 \text{ kg/m}^3$ at 4°C . There is usually a less dense upper layer and a dense lower layer. The region where the density changes rapidly according to the depth between these two layers is called the pycnocline layer.

3.3.5. Dissolved gases

There is a constant exchange of gases between the air and the sea. Therefore, all of the gases in the atmosphere are present in sea water. The most important of these are oxygen, carbon dioxide, hydrogen sulphide and nitrogen.

While the distribution of gases on the sea surface is provided by mechanisms such as waves, temperature change and diffusion, it is provided mostly by currents and turbulences in the deep. In addition, biological activities play an important role in the distribution of oxygen and carbon dioxide.

The sea surface is usually saturated with dissolved oxygen. Oxygen, contact of water with the atmosphere and photosynthesizing algae etc. produced by organisms. As you go down from the sea surface, the dissolved oxygen concentration decreases. Especially between 500-1,000 m, the oxygen rate drops to the minimum level. This change in oxygen is valid for phosphate and nitrate.

The high rate of CO_2 in sea waters is also present in the form of carbonic acid, carbonate and bicarbonate when its solubility increases depending on the pressure. Again, the nitrogen ratio changes depending on temperature and salinity and is found in the form of nitrates. As a result of the biological degradation of organic materials in sea water, carbon monoxide and methane gas also occur.

3.3.6. Particulate matter

Sea waters contain a wide range of particulate matter. The content of particulate matter in sea water; different mechanisms such as particles carried by rivers, dust carried by winds and volcanic ash act. In addition, there are also structural residues, feces, animal debris and particles from dead plants formed due to biological activities in sea water. Since the majority of these particles are

larger than 100 μm , they precipitate rapidly. However, particles smaller than 100 μm , such as algae cells and debris, accumulate near the surface as they precipitate rather slowly.

3.3.7. Optical properties

Suspended organic and inorganic substances and dissolved organic substances in seawater give seawater some optical properties. This causes the light to disappear by being absorbed and dispersed in sea water. Dissolved organic substances are effective in this disappearance. Red and yellow colours are absorbed rather than light, making sea water appear blue.

The depth at which sunlight can reach indicates the quality of the water. An instrument called a white painted Secchi disk with a diameter of 30 cm is used to measure this depth. In addition, measurements are made with light sources or photoelectric cabinets immersed in the sea.

4. DESALINATION OF SEAWATER (DESALINATION)

Today, the gradual decrease and pollution or absence of surface and underground water resources in some places has led humanity to use sea water.

Sea water, on the other hand, cannot be used directly in living spaces due to the chemicals and intense salt in it. However, it can be used after being purified by a desalination method and passed through various analyses and controls.

4.1. Historical development

Obtaining drinking water from sea water was first made on commercial ships in the 17th century. Sea water was evaporated with heat and then condensed to obtain drinking and utility water. The first facility was built on the island of Curaçao (Netherlands Antilles) in 1928. It was later made in Egypt in 1930 and in Saudi Arabia in 1938. These desalination plants included simple purification systems.

The use of sea water by desalination in the modern sense in the world has started since the first half of the 19th century. In this sense, a station that purifies sea water was established in Jeddah in 1894 for the Hijaz region, which experienced water scarcity and the need for water during the pilgrimage season became problematic. However, this station has become unable to meet the need over time and many new studies have been made for new facilities.

Countries with a coastline, where the need for water is starting to emerge day by day, have had to turn to projects to obtain fresh water from sea water. When we look at the countries with this orientation in general terms, the countries that have a coast to the sea and experience extreme drought have

started to benefit from the opportunities of converting sea water to fresh water. These countries are Saudi Arabia, United Arab Emirates, Kuwait, Libya, Qatar, Dubai, Iran, Spain, United States of America, Japan, Singapore, Malaysia and Italy.

Saudi Arabia is the world's largest desalination country with more than 30 desalination plants. Since 1990, Israel has found the opportunity to market the technologies it has developed to different countries of the world, in addition to producing solutions to the water problem, after the sea water desalination plants it has established on the Mediterranean coast. Greece follows all the innovations made in this field in the world by establishing many sea water desalination plants in order to meet the water needs of the southern regions of the country, to meet the water needs of its islands, to meet the water needs of the tourists coming to the country in summer and to be used in agricultural irrigation.

Due to the water shortages in some regions in Turkey in recent years, it has focused on the development of investments and projects for obtaining fresh water from sea water. For this reason, although not quite widespread, Avşa Municipality, Muğla Municipality and Istanbul Metropolitan Municipality have developed projects by making various researches and investments in this regard. Due to the fact that Balıkesir-Avşa Island does not have drinking water resources and is an intense tourism region, a sea water desalination project has been implemented by the local government. With its current water production capacity, the facility has guaranteed the fresh water need of Avşa Island until 2040.

4.2. Seawater desalination methods

4.2.1. Classical (Conventional) methods

There are classical (conventional) treatment methods from the past in order to bring the water that poses a risk to human and environmental health to the quality standards of drinking and utility water. These are aeration to bring oxygen to the water, disinfection to remove pathogenic microorganisms, settling in pools for coagulation / flocculation of colloid materials processed in fast or slow mixing units, and the use of filtration units for the removal of turbidity and unwanted chemical elements / compounds.

Treatment technologies for water taken directly from surface sources are generally these methods. However, in recent years, it has been seen that ozone for disinfection and activated carbon adsorption for advanced purification have been added to them. In units where groundwater is treated, such as springs or wells, the water is only chlorinated. It is then given to the water distribution system (network).

4.2.2. Chemical methods

Chemical treatment methods include ion exchange or liquid-liquid extraction processes. In general, these processes are found to be expensive in practice. However, ion exchange, which is one of these processes, is a special process used to produce high purity deionized water for special applications. Again, it is the most suitable method used for removing Boron (B) from sea water. It is not a practical process for waters with high dissolved solids content. It is a recommended method to be used together with the reverse osmosis process.

4.2.3. Distillation (Evaporation) methods

These are the methods in which thermal means are used to separate the changed state of water by using the state change feature of water such as gas or solid. The aim is to physically separate the water from the salt solution by evaporation and then collect it by converting it back to the liquid state. Thermal energy or solar energy is used for these methods. Before the 1980s, distillation methods were the most popular in seawater purification. In addition to being the first commercially used sea water treatment processes, these processes are still used extensively in the world.

4.2.3.1. Multi-Effect Distillation

In the Multi-Effect Distillation (MED) system, the taken sea water is evaporated by spraying it on the tubes through which the preheated water vapour passes, or by sending it to the surface of the tubes as a thin film. Subsequently, the water vapour formed is condensed by going through the second process.

This event is continued sequentially with a series evaporator called the process, and fresh water is obtained by condensation of the water. Depending on the size of a facility, there are 8-16 processors. This system is mostly used for industrial distillation processes. Despite being replaced by MSFD units over time due to cost and technical factors, interest in the system has increased in recent years and new facilities are being built.

4.2.3.2. Multi-Stage Flash Distillation

The Multi-Stage Flash Distillation (MSFD) method, developed in the 1950s, with the first plants established in the 1960s, is the simplest and most reliable desalination process, mostly used in large-scale capacity. Due to the advantages brought by its use, it has a very large share among the world's sea water treatment plants. Seawater is first heated to the highest temperature in a boiler. Hot sea water turns into fresh water with sudden condensation as it passes through stages, each of

which has a lower pressure and temperature than the previous one. While fresh water is collected in collectors, salt is pumped out in the form of concentrated brine.

4.2.3.3. Vapour Compression Distillation

Vapour Compression Distillation (VCD) is a method generally used for small and medium desalination units. Sea water is first heated with thermal or mechanical energy to obtain water. This water vapour is then compressed by the compressor and condensed in the heat exchanger using jet nozzles. This type of distillation plants are intensive and effective systems and are divided into two different subgroups in terms of heat source.

4.2.3.3.1. Thermal Vapour Compression Distillation

Thermal Vapour Compression Distillation (TVCD) is a thermal source of energy used to evaporate salty sea water. The sea water evaporated by passing through the heat exchanger is compressed with the help of a steam compressor and given to the system by jet nozzles and then condensed. As a result, sea water leaves the system from two different channels as fresh water and water with increased salt content.

4.2.3.3.2. Mechanical Vapour Compression Distillation

In Mechanical Vapour Compression Distillation (MVCD), mechanical energy is used instead of the thermal energy source in the heat exchanger. It is suitable to be used for desalination in small industrial units and hotels. When comparing thermal or mechanical vapour compression distillation processes, TVCD systems can have larger flow rates than MVCD systems. TVCD systems are multi-stage, while MVCD systems are generally single-stage.

4.2.3.4. Solar Distillation

The Solar Distillation (SD) process is based on the evaporation of water on free surfaces below its boiling point. It is a small scale hydrological cycle system. Its simple design resembles a greenhouse. The process is usually carried out inside a solar panel. The absorbed solar energy heats the sea water. The steam of the heated water is carried over the transparent cover with the circulated air. At this point, the condensed water is collected for use. The evaporation rate here is affected by factors such as the material of the area, the temperature of the water, the sun's radiation and the angle of inclination. Since the thermal energy required in this method is provided from solar heat, there is no energy cost. However, its dependence on climatic conditions is its most important disadvantage.

4.2.3.5. Rapid Spray Evaporation

The basis of the Rapid Spray Evaporation (RSE) method is that by spraying salty sea water into a chamber, the droplets evaporate rapidly and the dissolved salts accumulate at the bottom of the chamber. The evaporated water leaves the chamber and subsequently condenses. Although the technique looks promising and has been evaluated, no facilities appear to have been built except for a demonstration unit.

4.2.4. Membrane processes

The word membrane, which is a selective barrier and means allowing some things to pass while preventing others, is the general name of separator or selectively permeable materials in building materials.

The first permeable membrane was made by French Abble Nollet in laboratory conditions in 1748. In the last quarter of the 1800s, commercial production was opened and membrane technologies are widely used today with their continuously developed feature.

Membrane technology systems are widely used in the treatment of sea, lake, river and well waters and wastewater containing a wide range of dissolved substance concentrations and particle sizes. Especially in the last 30-40 years, there has been a great increase in the use of membrane technology systems. It has been an important factor in increasing the efficiency of reverse osmosis and a wide variety of filtration methods.

4.2.4.1. Microfiltration

In the microfiltration (MF) method, the separation process is somewhat similar to sieve. Suspended substances or colloidal particles larger than the pore diameter are retained by the membrane and separated from the water.

Since no chemicals are used in the application, toxicity problems are not seen in microfiltration. Although it can be used alone, it can also be used for pre-treatment in other methods.

4.2.4.2. Ultrafiltration

Ultrafiltration (UF) is a method used mostly for the removal of micro molecules and colloidal particles, depending on the pore size and structure of the membranes used. It is similar to microfiltration in terms of operation. It is very safe in terms of bacteria and virus removal. However, ion exchange is not possible.

Although it can be used alone, it can also be used for pre-treatment in other methods. In practice, besides the supply of drinking and utility water, it is also used to supply water for industrial activities such as food, milk, medicine, textile, chemistry, paper and leather.

4.2.4.3. Nanofiltration

Nanofiltration (NF) is a treatment method whose use has increased rapidly in recent years. With the nanofiltration process, it is possible to separate organic substances, dissolved compounds, calcium, magnesium and other ions from water. The most important feature of the membranes used is that they are ion selective. Although the majority of monovalent ions pass through the membrane, divalent ions such as sulphate and carbonate can be retained.

Surface water softening (removal of hardness) can be achieved with nanofiltration membranes. It is a method used in the recovery of wastewater in dairy farms, in the removal of organic matter and colour in the textile industry, in the concentration of organic substances in food and pharmaceuticals and in the removal of salt, and in the separation of difficult-to-decompose substances in wastewater before biological treatment.

4.2.4.4. Reverse osmosis

Osmosis is a natural phenomenon that occurs in nature on living things. It is the passage of one of two liquids of different concentrations with a permeable membrane between them. This process continues until both parties reach equilibrium.

Reverse osmosis (RO), on the other hand, is a membrane technology, and by applying a pressure greater than the osmotic pressure by the liquid with high concentration, the salts and organic substances in the mineral-rich liquid are transferred to the other side of a permeable membrane from salts and other minerals with reduced density is passed as a purified liquid. It is possible to filter about 99% of many minerals, bacteria and viruses in the water with this method.

This method is a widely used and developing technology in sea water treatment. It is a membrane desalination process that separates solutes from pressurized salty sea water. This type of reverse osmosis systems are facilities consisting of pre-treatment, high pressure pump, membrane assembly and post-treatment units.

The pre-treatment unit is to remove suspended solids and prevent salt precipitation or microorganisms from forming on the membrane. It generally consists of adsorption, electrocoagulation, microfiltration and coagulation / flocculation systems. High pressure pumps are units that pressurize salt water to pass through the membrane. Most of the energy consumed by the system is used here. Membrane assemblies are semi-permeable membranes produced in different configurations depending on the manufacturers, with the ability to pass fresh water by preventing the flow of salts in sea water. The final treatment unit is the unit where the water is removed from gases such as hydrogen sulphide and its pH is adjusted.

Membrane cost in the treatment with reverse osmosis method constitutes 20-30% of the total cost and 25-30% of the operating cost (membrane renewal). Therefore, the economy of the reverse osmosis method depends on the life of the membrane. Again, nearly half of the operating cost is energy expense.

Package type reverse osmosis systems, which are generally used for water purification in homes and sold ready-made in the market with all its elements, are very different in design.

4.2.4.5. Electrodialysis

Dialysis is the passage of molecules or ions across a membrane. Electrodialysis (ED), on the other hand, is a separation method in which positively and negatively charged ions in a solution are separated from the target solution by using an electric field to pass through permeable membranes to other solutions. It is very useful in the purification of sea water from mineral salts, compounds such as sulphate and nitrate, and in the recovery of wastewater.

4.2.4.6. Electrodeionization

Electrodeionization (EDI) is a purification method in which the anions and cations in the water are removed from the water by ion exchange resins and the integrated operation of electrical energy.

It is a technology in which pure water is obtained by using a combination of ion selective membranes and ion exchange resins trapped between two electrodes anode (-) and cathode (+) under a strong direct current. It is a proven and accepted technology for all industrial water treatment users requiring high purity.

4.2.4.7. Forward osmosis

Forward osmosis (FO) is a method in which permeable membranes are used to purify water, such as reverse osmosis. The membranes used here are more effective and efficient in removing unwanted pollutant parameters from water. As a natural process, forward osmosis requires less energy than conventional reverse osmosis method.

4.2.5. Hybrid membrane processes

Although the membrane technology used in sea water treatment has many advantages, it has some limitations. For this reason, a membrane project can be combined with a traditional process or hybrid processes can be made with a membrane process and another membrane process.

With hybrid membrane processes, it is possible to separate mixtures that are very difficult to separate with conventional processes. In addition, this type of

processes is preferred because it provides significant energy savings, high efficiency and productivity.

4.2.6. Ion exchange method

It is similar to the fact that plants combine the components they take from outside and produce something in their own body, or that the nitrogen in the fertilizer mixed with the soil is tied to the soil. This exchange is between the same charges, and only cations can be exchanged with cation exchangers and only anions can be exchanged with anion exchangers. Today, thanks to these ion exchangers, water hardness, salt content, nitrate, sulphate, organic matter and heavy metal ratios can be adjusted easily. The main ingredient of the ion exchangers in this method, which is widely used in the production of drinking water, is polymerization agents.

4.3. Comparison of desalination methods from sea water

The production of fresh water from salty sea water is constantly increasing as a result of falling costs with developing technologies.

As users of desalinated sea water in the world, Middle Eastern countries are in the first place with severe water scarcity. This is followed by America, Europe, Asia and African countries.

The facilities that produce nearly half of the amount obtained from the desalination of sea water are located in the Persian Gulf. More than half of these facilities are located in the United Arab Emirates. This is followed by Saudi Arabia and Kuwait. 17% of the water produced worldwide is produced in the Mediterranean. Spain is the largest producer in this region.

Looking at the distribution of seawater desalination technologies today, multistage flash distillation (MSFD) which is a thermal process and reverse osmosis (RO) plants, which is a membrane process, constitute more than 85% of the total facilities. Although these plants are approximately evenly distributed, the common trend is to prefer membrane processes. The advantage of the reverse osmosis method is that it takes up less space or small-scale facilities can be built and modularly. In addition, reverse osmosis membrane processes are considered to be more economical than traditional thermal processes such as multistage flash distillation (MSFD) and multi-effect distillation (MED).

The amount of energy used in reverse osmosis facilities is half of that used in other facilities. Because, in reverse osmosis method, mechanical energy is used as opposed to the thermal energy used to heat and evaporate the water, as in the thermal methods. This results in lower operating and maintenance costs.

However, in recent years, hybrid desalination systems combining thermal and membrane desalination processes are considered to be a good economical alternative. In this sense, hybrid membrane processes that combine multistage flash distillation (MSFD) and reverse osmosis (RO) processes come to the fore.

In addition to all these, multistage flash distillation (MSFD) method would be appropriate for a large-scale plant in regions with low fuel costs; it is suggested that reverse osmosis (RO) method would be appropriate for medium-sized facilities in regions with high fuel costs.

5. EXAMPLE OF AVŞA (BALIKESİR)

Avşa is an island located in the southwest of the Marmara Sea and has a surface area of 20.62 km². It is 4 nautical miles (approximately 7.5 km) to Marmara Island near Avşa Island and 18 nautical miles (approximately 33 km) to Erdek district.

The fact that Avşa is an island with limited water resources and its population, which is about 3 thousand with tourists in summer, reaches 50-60 thousand, causing serious water problems.

As a result of the cost analyses made among the options that came to the agenda to solve this problem, it was decided that the use of sea water would be much more economical by desalinating it.

The facility, which is the first in Turkey to obtain drinking and utility water from large-scale sea water, was completed and put into use in 2010. The facility consists of water intake structure, pre-sedimentation chamber, coarse and fine screens, raw water tank and pumps, pressure sand filter, cartridge filter, reverse osmosis membrane unit, dolomite filtering and remineralisation, disinfection and clean water tank. Avşa desalination plant operated by Balıkesir Metropolitan Water and Sewerage Administration (BASKİ) is shown in Photograph 1.



Photograph 1. Avşar (Balıkesir) BASKİ desalination plant

After being desalinated, the sea water with a salinity of 30,000 mg/L entering the facility is used with a salinity value of 5,000 mg/L. According to the calculations made by adding all the costs, the operating cost of the facility is around 0.4 \$/m³.

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Chapter 2

Importance of Length-Length and Length-Weight Relations in Freshwater Crabs

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INTRODUCTION

Despite the fact that there are over 6,700 known species of Brachyuran crabs, freshwater crabs contain more than 1300 species worldwide, belonging to eight families, and cover 20% of all brachyuran crabs. On the other hand, marine crabs have been considered as an important fishery product for many years around the world (Abbas et al., 2016:320; Johnston and Yeoh, 2020:527), but the importance of freshwater crabs has been ignored. For example, although freshwater crabs are widely distributed in the natural waters of many countries, very few studies have been conducted on biological properties such as exact species number, distribution of populations, length-length and length-weight relationships (Bandral, 2015:87; Aderinola et al., 2020:39; Fazhan et al., 2021). Therefore, it would be of great importance to increase the research on the determination of the length-length and length-weight relationships of freshwater crabs.

In many parts of the world, freshwater crabs live in tropical and subtropical areas (Yeo et al., 2008:275) and are considered to be one of the most ecologically important macro-invertebrate groups (Dobson et al., 2007:216). For example, freshwater crabs can be caught in many freshwater ecosystems, from clean, fast flowing stream currents to slow flowing rivers and streams, to freshwater marshes, stagnant ponds and rice fields, and even tree holes. (Cumberlidge et al., 2009:1666). On the other hand, it is known that some freshwater crabs, such as *Potamon savigny*, are found only in freshwater and can not survive in salty water for a long time. Moreover, it is also known that some of the freshwater crab species (i.e., *Parathelphusa* sp.) can survive for short periods in salt water (Yeo et al., 2008:275). Furthermore, terrestrial crab species can live long distances away from freshwater sources.

Freshwater crabs have become economically beneficial notably for approximately 20 years due to the below factors:

- Utilization of crab carapace as materials in medicine and pharmacy (i.e., chitin and chitosan production) (Rinaudo, 2006:603),
- Consumption by people as foodstuffs (Dobson, 2004:14; Aderinola et al., 2020:38; Jolaosho and Akintola, 2022:2),
- Wastes of crab processing plants used as feed additives and fertilizers (Bilgin and Fidanbaş, 2011:239),
- Keeping crabs alive in the aquarium as a hobby (Türkmen and Karadal, 2012:2824).

Apart from *Eriocheir sinensis*, other freshwater crab species are provided by hunting only from natural sources, although freshwater crabs have a great deal of

use, as noted above. On the other hand, in recent years, various methods have started to be used for the production of *E. sinensis*. For example, freshwater crabs are reproduced in shallow ponds, in soil ponds, in paddy fields, in small ponds and in cages in reservoirs (Sui et al., 2011:19). Depending on the increase in breeding activity of freshwater crabs, it is thought that the production of crabs will arise and the relationships between body length parameters in crabs caught from different populations will be of more importance (Aderinola et al., 2020:38). For that reason, in this review the importance and relationships of body length and weight parameters in freshwater crabs were studied.

IMPORTANCE OF LENGTH-LENGTH AND LENGTH-WEIGHT RELATIONS IN FRESHWATER CRABS

It is not possible to determine the ages of the shells of crab, lobster and shrimp shells that surround their bodies because of shell changes after molting. Therefore, relationships between body length parameters and weight are frequently used contrary to age dedication in crabs (Gülle, 2005:7; Ahmadoon, 2019: 3240; Syuhaida et al., 2019:223; Wanjari et al., 2021:82) However, relationships between body length parameters are taken into account for obtaining idea on below features

- .Comparison of males and females (Hegele-Drywa, 2014:800; Patil and Patil, 2012:14; Wanjari et al., 2021:85),
- Prediction of fecundity with development ratio and length in sexual ripeness (Udoh et al., 2011:145; Hegele-Drywa, 2014:814),
- Comparison of different species (Bandal, 2015:86; Abbas et al., 2016:319; Moslen and Miebaka, 2018:138; Aderinola et al., 2020:43; Fazhan et al., 2021: 107154-1),
- Comparison of different populations of same species (Torres et al., 2014:171; Bandal, 2015:86),
- Determination of condition factor (Patil and Patil, 2012:14; Moslen and Miebaka, 2018:191; Fazhan et al., 2021:107154-4).

DETERMINATION OF LENGTH – LENGTH RELATIONSHIPS

For this reason, below parameters are considered in crabs (Figure 1) (Micheli et al., 1990:496; Vannini and Gherardi, 1988:203; Syuhaida et al., 2019:226):

- Length of carapace (CL), abdomen (AL), claw propodite (PL),
- Width of carapace(CW), abdomen (AW), claw propodite(PW);
- Claw propodite height (PH)

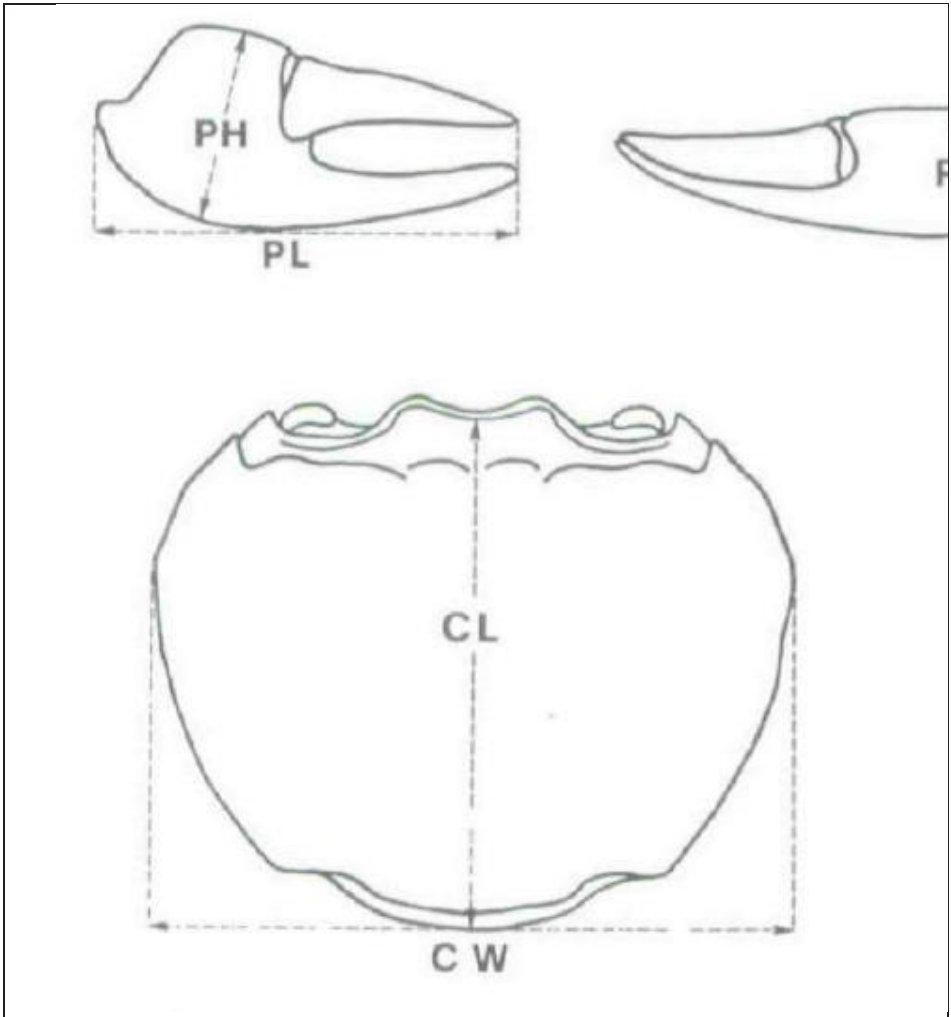


Figure 1. Taking body length parameters in crab (CL, CW, AL, AW, PL, PW, PH) (adapted from Micheli et al., 1990:493)

The relationship between lengths are found as following (Vannini and Gherardi, 1988:203; Thirunavukkarasu and Shanmugam, 2011:68; Wanjari et al., 2021:83):

$$\log y = \log (a) + b \log (x)$$

The "r" value is determined as a result of the regression analysis; if "b" is "3" it is isometric, if it is "less than 3" it has a negative relationship, and if "b" is "greater than 3" it has a positive allometric relationship.

OBSERVATION OF LENGTH - WEIGHT RELATIONSHIPS IN FRESHWATER CRABS

For this aim, relationships between CL–BW, CW-BW and/or AL–BW are used. In addition, to determine relations between body length and body weight following formula is used (Thirunavukkarasu and Shanmugam, 2011:69; Wanjari et al., 2021:86; Jolaosho and Akintola, 2022:8):

$$W = aL^b$$

$$W = BW \text{ (g)}$$

$$L = CL \text{ or } CW \text{ (mm)}$$

DETERMINATION OF CONDITION FACTOR (CF) IN FRESHWATER CRABS

Following formula is taken into consideration (Patil and Patil, 2012:13; Aderinola et al., 2020:40; Jolaosho and Akintola, 2022:11):

$$CF = [\text{Body Weight (g)} / (\text{Carapace Length (cm)})^3] \times 100$$

CONCLUSION

In conclusion, obtaining knowledge on the relationships between the body length parameters in crabs is a very important process in a scientific investigation (i.e., comparing distinctions between different crab species habitats, deciding corresponding development rate, contranstring different habitats of the species, utilise of morphological diagnostics in the systematic categorization of crabs). The greatest usually respected body length parameters for crabs are CL, CW and AW. Body lenght and BW relations are also crucial when crabs are commercially used. In addition, length–weight relationship of a crab species is taken into account to assess the available population biomass. Consequently, understanding the relationships both between body length parameters themselves and between body length parameters and body weight in crabs may have important inferences for crab farming and the governance of crab populations.

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Chapter 3

Leaf Water Potential Measurements For Irrigation Scheduling

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ABSTRACT

The use of plant-based measurement techniques in irrigation scheduling has been used extensively in recent years. One of the plant-based measurement techniques is the measurement of the leaf water potential value. Many national and international studies have been conducted on the use of leaf water potential measurements in irrigation time planning. As a result of the researches, suggestions were made for the leaf water potential values that should be started for irrigation application for many cultivated plants. There is a direct correlation between the quantity of water given to plants and the leaf water potential (LWP), according to studies. In this study, the steps to be followed in the measurement of leaf water potential values, previous studies were evaluated.

Key words: Water stress, leaf water potential, pressure chamber, cultivated plants

1. INTRODUCTION

Irrigation is defined as the supply of water, which is necessary for the normal development of the plant but cannot be met by natural means, to the root zone of the plant at the required time, in the required amount and in a controlled manner. The importance of this definition comes to the fore even more today, especially due to the decrease in the amount of water to be allocated for irrigation. Irrigation scheduling includes studies to determine when and how much irrigation water will be applied to a plant during the growing period. The basics of the main methods used for this purpose include monitoring the soil water content, observing the symptoms occurring in the plants and measuring the climate parameters. When and how much irrigation water to apply to plants can be determined by controlling the physiological symptoms caused by water stress in the plant using methods based on plant monitoring.

The water potential of a plant generally depends on the water content of the soil, atmospheric conditions and plant characteristics. The plant reacts to the environment it is in by making changes in some of its morphological features such as roots, stems and leaves. For this reason, direct measurement of vegetative parameters taken from various parts of the plant such as leaves and stems is one of the important methods used to determine irrigation time. One of these applications is the measurement of leaf water potential.

Leaf water potential (ψ_{Leaf}) is the most important metric used to quantify critical physiological processes, such as drought responses, and defines the plant's internal water status (Rodriguez-Dominguez vd., 2022: 2037). Leaf water potential (LWP), which defines the energy state of water within the plant, in other words, a driving force for water movement in plants; It is a negative value expressed as "bar" or "MPa" (1 atm=0.1 MPa=1 bar). An easy way to remember this is to think of water stress as a "deficit". The greater the stress, the more water deficiency the plant experiences (Shackel, n.d.). The variation of the specified value according to transpiration flow and soil water content shows the importance of leaf water potential in the evaluation of plant-water relationship.

Various methods (thermocap hygrometer/psychrometer, ShardaKov paint method, pressure chamber method and other methods) have been used since the 18th century to examine plant-water relations. Of these, the glass version of the device known as the "pressure bomb" was abandoned due to explosions (Dixon, 1914) and Scholander et al. (1965: 121), the steel chamber version was reinvented. Along with the developments in technology, the pressure chamber is the most preferred tool for determining the leaf water potential (ψ_{Leaf}) because

it provides an easy and safe measurement in field conditions (Rodriguez-Dominguez et al., 2022: 2038).

2. LEAF WATER POTENTIAL MEASUREMENTS

Today, there are functional differences between the pressure chamber and the instruments used to measure the leaf water potential (Pump-Up and Pressure Chamber). These; while human power is used to create pressure in the Pump-Up Chamber, pressure is provided by nitrogen gas in the Pressure Chamber. But in both tools, typically, a leaf is cut from a plant and sealed in the pressure chamber with the cut surface of the petiole exposed. The pressure is increased until a water drop appears on the cut surface of the handle. The pressure that provides the water outlet at the cut surface is equal to the leaf water potential.

The measurement of leaf water potential and the factors affecting the measurement are explained in detail below with the pressure chamber instrument (Pump-Up and Pressure Chamber), which has been widely used by both researchers and farmers in order to examine plant-water relations and to make irrigation programming of high-income crops (Yazdıç ve Değirmenci, 2018: 513; Bhusal et al., 2019: 536; Salbaş, 2020: 37; Bozkurt Çolak, 2021: 494; Rodriguez-Dominguez et al., 2022: 2038).

2.1. Factors affecting the leaf water measurements;

- **Measurement time:** As factors such as wind, relative humidity, temperature, radiation, soil depth, soil moisture content, soil temperature, soil texture, root growth change, the moisture potential of the plant also changes. Therefore, the measurement time should be evaluated carefully. Leaf water potential measurements can be made in two different time intervals as pre-dawn and mid-day. Predawn values generally reflect average soil moisture tension if the soil is uniform and irrigated effectively. Midday values reflect the stress experienced by the plant as it draws water from the soil to meet the atmospheric water demand.
- **Leaf sample selection:** Among the leaves of the plant to be measured, healthy leaves that are fully exposed to the sun, and that have completed their development should be selected and measurements should be taken from at least 2 leaves.
- **Observer:** The person making the measurement should observe the water output in the petiole well. It is important that the same person performs the procedure for the reliability of the measurements.

2.2. Measuring leaf water potential;

- A healthy, fully developed and sun-facing leaf is plucked from the plant (Figure 1a).
- Reading should be done immediately without waiting for the torn leaf. When there is a waiting state during the process, a new leaf should be selected and the measurement should be started again.
- Appropriate gaskets of different diameters are selected according to the petiole thickness of the plant (Figure 1b) and the cut surface on the stem of the torn leaf is corrected and placed on the cover with the help of gasket (Figure 1c).
- The leaf fixed to the cover is placed in such a way that the stem is outside the pressure chamber and the leaf blade is inside (Figure 1d).
- From the pressure source of the instrument, it should be applied by increasing the pressure until a water drop appears on the cut surface (Figure 2a and Figure 2b) on the leaf blade (Figure 1e).
- Pressure should be stopped as soon as water bubbles appear at the tip of the petiole. Otherwise, it may cause error in reading (Figure 1f).
- The pressure value that provides the water outlet on the cut surface is recorded as the leaf water potential value.
- Care should also be taken to record the weather (temperature, humidity, wind and other relevant weather changes) at the time of measurement to aid in correlating changes in readings and irrigation programming.
- In terms of occupational health and safety, the lid of the chamber should not be opened until the pressure is released after the measurement process is completed.



Figure 1. Leaf Water Potential Measurement with Pressure Chamber

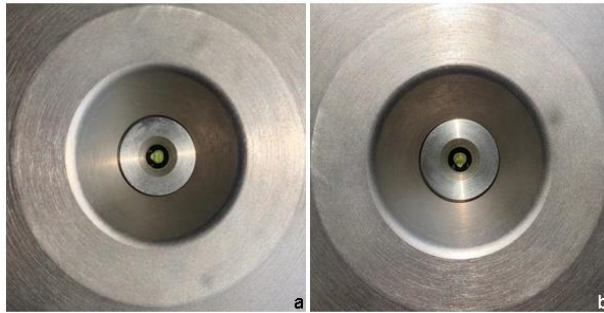


Figure 2. Change In Cut Surface as A Result of Pressure

There is a direct correlation between the quantity of water given to plants and the leaf water potential (LWP), according to studies. With regard to these studies, the leaf water potential values decrease as the quantity of water supplied to plants decreases (Torrecillas et al., 2000: 206; Demirtaş and Kırnak, 2006: 101; Maya and Kanber, 2008: 27; Köksal, Üstün and İlbeyi, 2010: 34; Yazdıcı and Değirmenci, 2018: 518; Alghory and Yazar, 2019: 71).

Many national and international studies have been conducted on the use of leaf water potential measurements in irrigation time planning. As a result of the researches, suggestions were made for the leaf water potential values that should be started for irrigation application for many cultivated plants.

Leaf water potential values in irrigation scheduling was determined as -17.8 bar for cotton by Maya (2007: 1), -13.0 bar for table grape by Bozkurt Çolak

(2010: 1), -14.0 to -18.0 bar for green dwarf beans by Köksal et al., (2010: 35), -15.0 bar for apple trees by Uçar et al., (2013: 429), -25.0 bar for pistachio trees by Aydın et al., (2014: 153), -13.27 to -21.76 bar for second crop corn and -14.24 to -19.43 bar for sorghum by Keten ve Değirmenci (2020:872), -14.5 to -15.7 bar for corn by Zavala-Borrego (2022: 414).

In Tekirdag, Turkiye conditions, sunflower leaf water potential measurements were carried out in the growing season of 2018 and 2019 for the planning of irrigation time to be applied in the flowering period, where the sunflower plant is most sensitive to water constraints (Salbaş, 2020: 37). Cantilever type pressure chamber (Scholander Pressure Chamber) device was used for leaf water potential measurements (Figure 3). Measurements were made before irrigation and at noon (12:00-14:00) when the sun's rays were perpendicular to the earth. In the measurements, the uppermost, sun-facing, young and fully developed leaves of the sunflower plant were used. Appropriate gaskets were selected for attaching the leaf to the pressure chamber to prevent gas leakage from the pressure chamber. The leaf selected for water potential measurement was cut off, its stem was cut and the leaf part was closed inside the room. Pressure was applied until water appeared on the cut surface. When the cut surface was covered with water, the pressure reading was taken as leaf water measurement. In measurements, Goldhamer et al. (1986) and Hisio (1993) approach has been used.



Figure 3. Leaf Water Potential Measurements for Sunflower Plant

The leaf water potential values for the sunflower plant obtained from the treatment subjects varied between -10.6 and -13.3 bar for 2018, and between -12.5 bar and -16.1 bar for 2019. The LWP values obtained depending on the amount of irrigation water applied among the treatment subjects changed, and the leaf water potential values decreased with the increasing water constraint. The lowest LWP values were obtained from the experimental subject that did not apply irrigation water (0%), and the highest LWP values were obtained

from the subject that applied the most irrigation water (125%). Linear relationships were obtained between the leaf water potential values measured from the treatments and the seasonal evapotranspiration. The obtained values were found to be statistically significant at the $p < 0.01$ level in both years. In the same way, linear and $p < 0.01$ relations were determined between the leaf water potential values obtained from the treatments and the sunflower grain yields. According to these results, it can be recommended to use -13 and -14 bar leaf water potential values for irrigation of sunflower plants, especially in limited water supply conditions. Sionit and Kramer (1976: 540) irrigated the sunflower plant in their study conducted in the USA when the leaf water potential values reached -16 bar and -23 bar. As a result of the research, they reported that the sunflower plant entered water stress when the leaf water potential value fell below -16 bar.

3. CONCLUSION

The use of plant-based measurement techniques in irrigation scheduling has been used extensively in recent years. One of the plant-based measurement techniques is the measurement of the leaf water potential value. In particular, the correct determination of the leaf water potential threshold values is important in terms of the conservation of water resources and the production values to be obtained, in order to determine the right time to start irrigation of the cultivated plants. In this study, the steps to be followed in the measurement of leaf water potential values, previous studies and the results of the research carried out on the sunflower plant in Tekirdag conditions were evaluated. It is thought that the information given will be useful to producers and experts who will conduct research on the subject.

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Chapter 4

Investigation Of The Structural Features Of Dairy Cattle Facilities In Bursa Region

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ABSTRACT

INTRODUCTION

When the increasing population rates in the world are evaluated, the reality of how important nutrition and food sources are. Although animal products, which are among these food sources, have an important place in human nutrition, the amount of animal food per capita in Turkey remains at low levels compared to other OECD member countries.

While the production of milk and dairy products, which are necessary at every stage of human life, was 624,285 tons and 8,67 kg per person in 2010 in Turkey, it reached 1.195.940 tons in 2022 and realized as 14,23 kg per person (TUIK, 2022). Despite this increase, while the average milk consumption in European Union countries was 5,8 kg/year in 2019, this rate is only 18,1 kg/year in Turkey. Likewise, when we look at the cheese consumption in 2019, the average cheese consumption in European Union countries is 19,1 kg/year, while this rate is quite low with 8 kg/year in Turkey (Anonymous, 2020). According to TUIK data for 2021, the total number of cattle in Turkey is 17,850,543 heads.

Animal husbandry is of great importance in the agricultural sector. With the increase in population, the demand for products such as meat and milk, which are important for human nutrition, is increasing. This increasing demand and need can be met by productive animal husbandry in modern enterprises and facilities, the structures of which are regularly planned and optimized in terms of environmental conditions.

When shelter and environmental conditions are mentioned, climatic, structural and social factors are understood (Mutaf and Sönmez, 1984). Climatic factors are ambient temperature and relative humidity, air velocity, various gases and dusts. Structural and social factors are resting places and stops, feeders, transition points, drinkers, walking areas, group sizes, and building elements depending on the shelter floor arrangement (McFarland, 2000; Rodenburg, 2003). Shelters require proper planning to protect animals from climatic environmental conditions and to provide an environment suitable for animal welfare and wishes. Shelter temperature and relative humidity values should be in accordance with animal requests. Otherwise, the health of the animals will be adversely affected and the desired yield will not be achieved. (Yüksel and Şişman, 2015).

Shelters are planned in order to eliminate the negative effects of environmental conditions for animals and to provide efficiency in production by creating a welfare environment. In the selection of shelter; Soil structure, topographic features of the land, location to the road, accessibility to water and electricity, location of other structures in the enterprise and the way they are arranged (or usefulness of the structures) should be taken into consideration. Base arrangement and technology use should be provided to increase the work

efficiency of the employees in the shelter. Appropriate development areas should be left for the shelter to be enlarged by considering future developments. The shelter should be placed in such a way as to allow integration with other structures in the operation center. It is expected that the construction of the barns is simple, air inlet and outlet openings are sufficient, comfortable and suitable for versatile use (Ekmekyapar, 2001; Arıcı et al, 2014).

Different barn models are applied in dairy cattle breeding in Turkey and in the world. The majority of small-capacity and traditional production methods use the barn type with closed and tied stalls. The possibilities of such enterprises to expand their capacity and implement innovations are very limited. These shelters are very difficult to operate. Feed distribution, manure cleaning, milking and maintenance are time consuming and tiring. Since animals are constantly connected, it is very difficult for them to fulfill their natural behaviors, that is, to ensure animal welfare. In addition, an unsuitable working environment for the caregiver reduces productivity. The free-open (semi-open) barn model, designed in accordance with the natural behavior of dairy cattle, allows animals to move freely. This type of barn is arranged according to the climatic conditions of the region and is applied in such a way that the east and south facades are open or completely open. Resting areas are freely provided for animals, and walking areas and mangers are arranged outside the barn. This building model, which was designed by taking into consideration the cost of the building and animal welfare, also has various disadvantages. First of all, there is a constant use of litter in the resting place, especially in winter. Since at least one side of the barn is open, yield losses can be seen as regional winds directly affect the animals. Therefore, we may encounter effects that restrict the widespread use of this barn model (Arici et al., 2014).

The ambient temperature of the shelter should be located within the limits called the Comfort Zone, where the animals perform their productivity in the best way and provide their comfort. In the researches, it has been determined that the optimum temperature value for dairy cattle is between 4 and 21 °C. At temperatures above this value, heat stress occurs depending on the height in air humidity. In particular, Temperature-Humidity Indicators are used to determine the effects of temperature and humidity on dairy cattle (Armstrong and Welchert, 1994). In order to minimize the decrease in efficiency caused by heat stress, it is recommended to provide good ventilation in the shelter by natural means, to use mechanical fans to increase air circulation, and to cool by sprinkling (Armstrong, 1994). This efficiency can be increased with the insulation to be provided in the building elements (Bickert et al., 2000).

One of the climatic environmental conditions in dairy cattle barns is relative humidity. Relative humidity has a significant effect on animals and its negative effects become more evident with temperature. Since high relative humidity and temperature stress the animals, especially in summer, it leads to a decrease in feed consumption and decreases milk yield. For this reason, the most suitable environmental conditions for dairy cattle can be provided at temperatures of 10-15 °C and relative humidity of 70-80% (Balaban and Şen, 1988). Likewise, the combined negative effects of high temperature and relative humidity and high solar radiation are more common. As a result, yield losses occur as feed requests and feed consumption decrease (Mutaf and Sönmez, 1984).

In the welfare and productivity of animals, good air quality must be ensured in the barn environment. There should be good ventilation in it. In animal shelters, ventilation is carried out in order to provide the oxygen requirement of animals, to remove various harmful gases and microorganisms, to provide heat and water vapor balance (Noton, 1982). For this purpose, dairy cattle barns can be named as closed and open systems and as cold, adjustable and warm barns according to their insulation properties (House and Rodenburg, 1994).

Marmara region is one of the regions where dairy cattle breeding is intense. Likewise, the basic production structure of many enterprises in Bursa and its districts is based on dairy cattle breeding. However, the majority of enterprises have traditional production methods and structural problems that make production and efficiency difficult in many ways. In this study, the structural problems of dairy cattle enterprises in the settlements in Bursa Province, Nilüfer District, Karacaoba Neighborhood were examined. The problems for the newly established businesses in the region were analyzed on-site and solutions were developed.

MATERIAL AND METHOD

The research was carried out in dairy cattle farms located in Karacaoba Neighborhood, located within the borders of Nilüfer district of Bursa province. The region is located in the northeast of Uluabat Lake. The boundaries of the neighborhood are 2,3 km in east-west direction and 4,6 km in north-south direction. The entire area is 7,141,493 m². In the study, businesses within the borders of Karacaoba Neighborhood were examined (Figure 1).

According to 2019 data in Nilüfer district, it has been determined that there are 12836 cattle, 3791 of which are milked cows. With a milk yield of 9.53 kg/day per animal, total milk production was 13,013.53 tons/year. In Turkey, the total number of cattle is 17,850,543 heads, according to the 2021 TUIK data. Bursa's annual average temperature is 14.6 degrees, annual average precipitation is 709.5

mm, and average humidity is 73.16%. The annual average wind speed in the region is 15 km/h and the prevailing wind breeze is in the north-northeast direction. Bursa's annual average temperature value is 14.2 degrees, annual average precipitation is 674 mm, humidity average is 69%, although the prevailing wind direction is northeast, it varies and the annual average is 12,08 km/hour (Anonymous 2022).



Figure 1. View of the Research Area

Due to the lack of statistical data on the financial values, agricultural incomes and land sizes of dairy cattle breeding enterprises in the research region, 15 randomly selected enterprises were chosen as research material, taking into account their size and location in the selection of enterprises. In this election, businesses are classified as small, medium and large scale.

The data on the business structures determined as research material were provided with measurements, sketches, surveys, data obtained from the relevant municipality and photographs taken (field and drone photos). All structures and structural elements of the dairy farms in the farm yard were measured with laser meters and sketches were drawn in computer-aided programs (CAD) at the scale of the situation. Structural features of the shelter and other structures are presented with views, sections and photographs. The structural characteristics of the examined enterprises were evaluated by filling in the questionnaire forms prepared for the buildings according to the results of one-to-one interviews with the owners, employees and veterinarians.

RESULTS

The average dairy cattle capacities of the 15 enterprises examined in the research vary between 10 and 180 heads. Animal capacities may vary depending on the structural size of the enterprise, the financial possibilities of the owners of the enterprise, and livestock and economic developments. Although most of the surveyed businesses are family businesses, there are businesses with employees. The majority of the surveyed enterprises are over the age of 15, and only one enterprise has been established in the last 5 years. Again, the ones other than the last established enterprise were not built in line with any project. It has been observed that the businesses are mostly done with traditional methods. Project enterprises were built without any support from any institution or organization.

Most of the enterprises produce especially fodder crops to meet their roughage needs, and purchase the missing part from outside, and use this mostly by supplementing it with their own feed. Small-scale enterprises, especially in the north of the study area, far from the settlement, cultivate forage crops near the enterprise, so that they can reduce their feed costs, whose costs are about 2 times compared to the previous year at the date of the examination.

Briquettes and bricks were used as the wall material of the animal shelters in the enterprises, and it was determined that 2 enterprises were made of wood material. In 3 buildings, it was determined that the shelter was covered with sheet metal panels instead of the wall (Figure 2).



Figure 2. Shelter side wall material preference (sheet material)

It was observed that wood was used in the bearing elements of the old buildings, steel was used in 6 enterprises, and reinforced concrete was used in the new and large-scale structures. Plaster-paint or any coating and heat insulation

materials were not used in the examples where brick was used on the side walls of the barn structures (Figure 3). This not only reduces the life of the building but also affects the animal welfare due to the fact that the structure is exposed to thermal changes in summer and winter.

The barn roofs are covered with insulated sandwich panel material in 2 shelters and with uninsulated tile, corrugated and single layer sheet material in the others (Figure 4). The uninsulated roofs cause heat losses in winter, while in summer it can lead to overheating of the shelter.



Figure 3. A view from the shelter exterior



Figure 4. An example of a shelter roof covering

Natural ventilation system is applied in all of the barns. The inadequacy of the air inlet and outlet openings required for natural ventilation cannot provide the desired air exchange. Only two of the barns examined have ventilation openings

on the side walls and in the ridge above the roof. In the vast majority of them, ridge ventilation was not provided and air inlet openings were not left on the side walls, and the doors were found sufficient to be used for this purpose. In the barns where the free barn system is used, one surface is kept completely open.

The ventilation system plays an important role in ensuring animal and employee health in the shelter interior. With ventilation, the accumulation of harmful gases caused by animal respiration and feces in the shelter is prevented, and it becomes possible to maintain the indoor temperature and humidity balance. When a suitable environment is provided for the animals, an increase in feed consumption can be achieved and this situation will directly reflect on the animal yield. High temperature causes stress in dairy cattle and reduces productivity. For this reason, it is important to choose the cold barn type, which is designed by arranging the air inlet and outlet openings by leaving continuous openings in the side wall and ridge, in accordance with the characteristics of the region, in the shelter planning, in which maximum ventilation in summer and minimum ventilation in winter can be made. (Olgun, 1989; Arıcı et al., 2014).

The barns of the examined enterprises were built in 3 different types. 3 barns with free stalls, 3 with tie stalls and the other 9 barns are planned as free barns, which were built recently and evaluated among large-capacity enterprises. Although the business structures, which have been in use for about 20 years, were designed in a tied system at first, most of them were converted to a free system by removing the fastening mechanisms. Here, the producers stated that they took the developments in the region into account or made changes in their structures with the warning of the experts. However, in the observations and measurements, it has been observed that the areas reserved for animals are insufficient (the area allocated per animal is between 2.5-3 m²), therefore the animals may experience stress in the shelter. In some barns, it was observed that there were quite large areas due to the low number of animals. It is stated that in this type of shelters, an area of 5-7 m² per animal should be allocated at the resting place (Arici et al., 2014). In addition, as seen in Figure 2, the side wall height of the barn is 2.3 m, which is quite insufficient considering the climatic conditions of the region, which can affect the indoor air volume and thus the air quality. Yüksel and Şişman, 2015, suggest the sidewall height as 2.55 m in temperate regions.

It is seen that large-capacity enterprises are planned as free-stall barn type. It has been determined that these enterprises were built without benefiting from any support, and they benefited from the personal efforts of the business owners and the plans of the businesses they saw in the environment. It can be said that they have a clear advantage over the traditional businesses. The most important indicators are that the level of mechanization in the shelter is better than in other

enterprises. Feed distribution, manure cleaning and milking automation are developed. Herd management and feed ration are carried out in a planned manner. Animal needs are given priority. Animals are raised by constantly consuming the feed given in their shelters and keeping them under control. Since the floor arrangement of traditional farms does not leave enough space for animals, it is insufficient to provide the desired environmental conditions. In the barn, the feeders are not arranged properly, the drinkers cannot provide water to the animals continuously in some enterprises (Figure 5).



Figure 5. In-house drinkers

Appropriate space has not been allocated for maternity chambers and newborn animals. This may result in injury or even death of newborn animals. In addition, most of the farms do not have a calf compartment and the animals are raised collectively.

In most of the 67% of the enterprises, there is no fertilizer and feed storage structure. Manure, most of which is shoveled by hand, is transferred out of the barn, where it is collected for a certain period of time and transported to the fields (Figure 6).



Figure 6. An example from the outdoor manure storage area

Significant pollution has been observed in this process. The level of flies, insects and odor has the potential to harm the environment and animals. It has been observed that even in large enterprises, there are insufficient fertilizer warehouses or significant problems are encountered due to planning errors. Similarly, feed storage structures either do not exist or are not of sufficient size and feature. Silage is mostly stored horizontally above the ground. It has been observed that it is also stored in trailers in some enterprises. Concrete silos are only available in businesses that have been built recently. Poor storage can lead to large feed losses.

In most of the structures, serious corrosion has been observed in steel carriers, roofing materials and even reinforced concrete parts. In the building example, the deformation of the steel roofs is at an advanced stage and it has been determined that the structure and the living things in it have reached such dimensions that they can be damaged, and the owner has stated that he will make repairs in these areas (Figure 7).



Figure 7. Example of corrosion in structural elements

Finally, many businesses are located in the neighborhood residential area. This causes businesses to be stuck in limited and small areas, not suitable for correct planning and mechanization, and completely limited growth opportunities in the future. At the same time, these businesses, which are located in the residential area, disturb the people living in the houses in terms of smell and pollution (Figure 8).



Figure 8. Businesses intertwined with residential areas

CONCLUSION

In the study, the structural features and problems of dairy cattle farms located in Bursa Province Nilüfer District Karacaoba Neighborhood were tried to be determined. Although it is seen that the enterprises over 20 years old and built with traditional methods in the study area have many problems, it is seen that the region has a very important potential for dairy cattle breeding due to its advantages such as its past habits, proximity to metropolitan cities, and the presence of many dairy processing facilities. It is possible that the problems encountered in the enterprises can be improved with the renovation works. However, the renewal of the enterprises developed in the traditional settlement order and the increase in their capacities limit the desired arrangements due to the inadequacy of the courtyard areas. However, it would be more appropriate to establish new businesses with the relocation of businesses outside the existing settlement center.

In the study, the majority of existing enterprises are insufficient to provide animal productivity. The most important indicators of this are that barn systems suitable for animal welfare are not used. Since the dimensions of the majority of the shelters are not suitable for animal demands and adequate ventilation is not created, the air quality of the shelter affects the animal productivity. The most

important indicator of animals' adoption of their environment is their adaptation to the weather conditions in their environment.

Barns are planned in order to provide suitable environmental conditions for animals, to increase animal productivity by improving health conditions, to create a suitable environment for workers and to extend the life of the building by preventing damage to building elements (Olgun, 1989). Dairy cattle are more productive in comfortable environments. In order to ensure the positive development of the behavior of animals during feeding, movement and rest, barns should be clean and dry and protect animals from variable weather conditions (Holmes and Graves, 1994).

In order to ensure animal welfare, the environmental conditions in the shelter should be controlled according to animal requests. In this context, subjects such as temperature, relative humidity, air flow, chemical composition of the air, illumination, and noise should be carefully considered (Yaganoglu, 1988; McFarland, 1994).

The most important indicators that determine the profitability of a business are the investment cost and operating expenses. With the barn design that can be arranged in accordance with the regional conditions, the operating investment cost can be reduced. For example, semi-open barn systems not only provide good ventilation, but also reduce construction costs due to less material use and lightweight construction. Tie-stall barns increase the construction cost per animal. Şimşek and Yashioğlu, 2006, determined that greenhouse type shelters will be built at 57% lower cost compared to closed system shelters. As a result of the effective use of mechanization processes in the barn, which increase work efficiency, in reducing the operating costs, especially since it will facilitate feed distribution, manure cleaning and milking, it will be possible to get a return on the investment made beforehand.

Barns should be designed in a way that will meet animal demands and natural behaviors, taking into account the choice of location and future developments, with minimum use of labor and low cost, and it should be ensured that the problems that may be encountered in the future are at a minimum level. Otherwise, it should be noted that it will be very difficult and costly to arrange the mistakes to be made. For this reason, dairy cattle business structures should be designed by taking into account the opinions and suggestions of experts.

In dairy cattle breeding, the environmental conditions of the animals have an important effect on the productivity of the animals along with the desire and welfare of the animals. The shelter environment must first have good air quality. For this, attention should be paid to the planning of structural features, from building insulation to sizing the ventilation system. Considering the age and

characteristics of the animals, a suitable barn floor arrangement should be made that will enable the animals to find a comfortable environment.

The wishes of the employees should never be ignored, and the use of automation and herd management systems that will ensure work efficiency should be supported. Low cost and easy installation of the planned barns should be provided to meet the animal and employee demands. In summary, the following suggestions can be made to eliminate the problems observed in the research area;

- Measures should be taken to prevent environmental and water pollution,
- The contribution of the young population to production should be increased,
- Systems for low-cost building design should be evaluated,
- Shelters that increase the welfare of animals and workers should be developed,
- Producers should benefit from appropriate business support,
- Feed storage and fertilizer warehouses should be established,
- Producers should be provided with information about new production techniques,
- Product sales prices should be increased while input costs are reduced,
- Producers should be provided to increase their quality production and sales opportunities in an organized manner,
- Efforts should be made to reduce feed production costs.

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Chapter 5

The Status Of Livestock Production In Terms Of Global Warming And Its Future Perspective

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ABSTRACT

INTRODUCTION

In recent years, extreme droughts and extraordinary weather events such as hurricanes and floods have made it necessary to address the problem of global warming more seriously. Greenhouse gases, the rate of which is increasing day by day in the atmosphere, especially due to human-induced effects resulted from industrialization, cause global warming to accelerate.

The global average annual temperature rose 0.94°C in 2016, at an unprecedented rate of increase for a long time, compared to the 20th century average of 13.9°C. (NOAA, 2017). The global mean surface temperature will be expected to increase within 0.3°C to 4.8°C till 2100 based on IPCC's Fifth Assessment Report (IPCC, 2014). According to the report prepared by the IPCC in 2018, global warming is predicted to reach 1.5°C by 2040. It has been reported that if this prediction comes true, the already scarce water resources will gradually be depleted, sea levels will rise due to the rapid melting of glaciers, and famine and migration problems will arise due to the depletion of food stocks. (Öztürk and Öztürk, 2019).

According to the IPCC's perspective, climate change can lead to various environmental problems such as drought, flooding and health risks. Ambient temperature, light period, and precipitation are various direct effects of climate change. Furthermore, indirect effects include disease occurrence, reduction in feed and water resources, and reduction of existing pasture. It is expected that both direct and indirect effects of climate change will cause various negative effects on the productivity of livestock. Both well-known scientists who contributed to the IPCC's 4th Assessment (AR4) report and the results of the analysis on the evolution of the temperature-humidity index (THI) in the Mediterranean region in the last fifty years of the 20th century, provide satisfactory information on urgency to follow the global warming theory (Rashamol et. al., 2018).

Global warming will not negatively affect everywhere in the world. Thornton et al. (2007) predicts a slight increase in crop yield at mid and high latitudes for a 1–3 °C increase in local average temperature. In addition, frosts, heat waves or heavy rains in these regions can deprive the advantages of temperature increase. However, a lower temperature rise of around 1-2 °C may adversely affect crop production and grain production at lower latitudes. The most affected areas will be in the northern hemisphere, particularly in North America, northern Europe, northern Asia, and the Mediterranean basin and West-Central Asia which are located at a lower latitude (Easterling et al., 2007).

RELATIONSHIP IN GLOBAL WARMING AND LIVESTOCK

According to the United Nations, the world population is approximately 7.6 billion in 2017. Twelve years ago this figure was 6.6 billion. Although this growth is slower than 10 years ago, with an annual average increase of 83 million people the world population will reach 8.6 billion in 2030 and 9.8 billion in 2050. Population growth, urbanization and income growth in developing countries are the locomotives of increased demand for livestock products (UN, 2017).

For meat production, approximately 65 billion chickens, 1.5 billion pigs, 1 billion goats and sheep, and 330 million cattle and buffalo are raised worldwide (FAOSTAT, 2016). It is stated that the dairy cows are approximately 234 million and there are 7.6 billion laying hens in the egg production sector. Fifty percent of the global agricultural gross domestic product is produced by the livestock sector (Herrero et al., 2016). Livestock sector also supports the livelihoods and food security of approximately 1.3 billion people in developing countries (FAO, 2017a). Global demand for animal products is expected to double by 2050 as the worldwide standard of living is improving day by day. On the other hand, climate change menaces a threat to livestock due to its effects on forage crops and forage quality, water supply, livestock and milk production, animal diseases, animal reproduction and biodiversity (Rojas-Downing M. M., 2017). The effects of climate change are firstly due to increase in atmospheric CO₂ concentration and in temperature, the change in precipitation regime and a combination of these factors (Aydinalp and Cresser, 2008, Henry et al., 2012, IFAD, 2010, Nardone et al., 2010; Polley et al., 2013; Reynolds et al., 2010; Thornton et al., 2009).

The effects on livestock will vary according to the region, animal and production type and may have positive or negative effects (Sejian et al., 2017). For example, Northern Europe is already showing signs of a warmer and wetter climate, which increase the expectations for positive trends in plant and pasture development (Stagge et al., 2017) and projected climate changes are expected to have a positive impact on forage yield per hectare in Central Europe (Gauly et al., 2013). Production decreases are expected in other regions dominated by winter precipitation-based production, such as South Australia (Howden et al., 2008) and southern regions of South Africa. Some countries in these regions have very high livestock densities, and livestock production mostly composed of industrialized livestock systems engaged in raising pigs, poultry and dairy cows. Indirect effects of global warming such as soil infertility, water scarcity, deterioration of grain yield and quality, and the spread of pathogens may disrupt livestock production in these systems more than direct effects. Indeed, in these systems, livestock can better cope with the direct effects of high temperature, i.e. heat stress, with the help of feeding arrangement, cooling techniques or farm

management. On the other hand, the technical measures used to adapt the air temperature of the barns to the comfort range of the livestock cause higher energy consumption, thus worsening global warming and increasing the overall costs of livestock production. In addition, industrialized systems produce more fertilizer than fertilizer needed in nearby cultivated fields, causing the accumulation of phosphorus, nitrogen and other pollutants in the soil (Thorne, 2007).

Livestock systems based on grazing and mixed farming systems should be expected to be more affected by global warming than an industrialized system. It will be resulted from the negative impact of lower precipitation and more drought on the healthy development of plants and grasslands, and the direct effects of high temperature and solar radiation on livestock. (Rashamol et. al., 2018).

Livestock contributes significantly to increased CH₄ and N₂O concentrations in the atmosphere. In general, two-way effects on climate change is existed, namely contribution of livestock production to climate change and the impact of climate change on livestock. Therefore, improving livestock production under the changing climate scenario should aim to both reduce greenhouse gas (GHG) emissions from livestock and the impact of climate change on livestock production. Adapting to climate change and reducing greenhouse gas emissions may require significant changes in production technology and agricultural systems that can affect productivity (Sejian et. al., 2015).

Greenhouse gas emissions directly from livestock are CO₂ as well as methane (CH₄) resulted from enteric fermentation and manure management, and nitrous oxide (N₂O) resulted from nitrogen deposition by manure management or application to pastures and cropland. Methane has a much shorter lifetime (about 12.4 years) in the atmosphere than CO₂ (hundreds to thousand years) but has a much higher warming efficiency per molecule and per kg (Reisinger ve Clark, 2017).

Methane, mainly generated by enteric fermentation and manure storage, is a gas that affects global warming 28 fold than carbon dioxide. Nitrous oxide, resulting from manure storage and the use of organic/inorganic fertilizers, is a molecule with a global warming potential 265 times greater than carbon dioxide. Carbon dioxide equivalent is used as a standard unit in order to reveal the global warming potential more clearly. (IPCC, 2013). Beef cattle (45%) and dairy cattle production (26%) have the highest greenhouse gas emission potential among the livestock production types (Table 1).

Table 1. Greenhouse gas generation rates from enteric fermentation and manure storage by animal species, based on 2010 data

Livestock	Enteric Methane (%)	Manure Storage Methane (%)	Manure Storage Nitrous oxide (%)	Total CO ₂ Equivalent in Gigaton
Beef	91	3	6	1,8 (% 45)
Dairy Cattle	85	8	7	1,0 (% 26)
Bison	91	2	7	0,5 (% 12)
Sheep	93	3	4	0,2 (% 4,5)
Goat	93	4	3	0,2 (% 4)
Poultry	0	34	66	0,1 (% 1,5)

FAO, 2017b

Most of the production in ruminant farming, which is indisputably one of the most vulnerable food and fiber production systems to climate changes, is done in large pastures where inputs are low and feed production and water supply vary according to seasonal climatic conditions. Global feed production for all livestock requires 2.5 billion hectares of land, but about 90% of animal protein comes from feed produced on 2 billion hectares of grassland, of which 1.3 billion hectares are not arable (Mottet et. al., 2017).

SITUATION IN TURKEY

According to the World Meteorological Organization (WMO), 2010 was the hottest year in Turkey with a temperature increase of 2.0°C, and 2018, with an average temperature of 15.4°C, was recorded as 2nd hottest year since 1971. This recorded temperature of 15.4°C is 1.9°C higher than the climate normal (13.5°C average temperature) within the 1981-2010 period (Anonymous, 2019).

Turkey's total greenhouse gas emissions in 2017 increased by 140% compared to 1990 and reached 526 million tons, of which 62.5 million tons were greenhouse gas emissions from agriculture, which increased by 37% in the same period (Dellal et. al., 2020).

According to the Environmental Performance Index (EPI) created in 2002 to evaluate the situation of countries against global climate change, Turkey ranks 99th among 180 countries with a score of 42.6 in 2020 (Wendling et. al., 2020). In the same report, Turkey ranks last in Eastern Europe, which includes 19 countries, in the ranking made according to the Ecosystem Vitality Score, and ranks 139th with Sri Lanka with 36.9 points in the general ranking.

The climate scenarios prepared by the IPCC in 2001 reveals that a hot and dry climate awaits Turkey in 2030, temperatures are expected to increase in both winter and summer seasons, the increase in winter and summer temperatures will be 2°C and 2-3°C, respectively (Kadioğlu, 2007).

According to the A2 climate simulation, it is estimated that temperatures in Turkey will increase 1.0°C–2.5°C within the period of 2041-2070, and 2.5°C–5.0°C within the period of 2071-2099. It is expected that there will be significant changes in the regional distribution of annual precipitation, and the annual precipitation amount, which will increase in the northeast of Turkey, is expected to decrease in the south (Şen, 2013).

Yaslioglu and Ilhan (2018) calculated the temperature humidity index (THI) for broiler chickens in the Marmara Region using 40 years of data covering the years 1974-2013. By analyzing GIS-based THI maps, created based on calculated THI values, they determined that 21°C, which is the threshold value for broiler chickens, is exceeded during the summer season in all places except Bursa, Balıkesir, Sakarya, Black Sea coasts and southeast Marmara.

Koç and Uzmay (2019) determined that climate change will lead to a 10-50% cost increase in the production costs of dairy cattle farms in Turkey until 2044 of which 48-71% heat stress and 24-52% increase in feed prices.

IMPROVEMENT MEASURES

Increasing environmental problems have caused the concept of sustainability to be taken into account more in the agricultural sector as in all other sectors in recent years. For agriculture/livestock systems, the concept of sustainability means maintaining production levels not exceeding the capacity of the ecosystem that supports it, or in other words **“meeting the needs of the present without compromising the ability of future generations to meet their own needs”** (Duru and Therond, 2015).

According to Kaufmann (2015), the following mitigation strategies should be adopted to cope with the climate change impact of livestock: **a)** improving production efficiency, reproduction and animal health, **b)** using manure management practices to recycle and recover the nutrients and energy contained in manure, **c)** supplying low-emission inputs such as feed, **d)** use of feed additives such as amino acids, enzymes, and gut modulating products such as prebiotics and probiotics, organic acids and phytobiotics, and **e)** adoption of new technologies (Tullo et. al., 2019).

Projected increases in extreme weather events and changes in feed supply, composition and quality, and animal feed rations will affect animal product supply and food supply. These effects are likely to be in developing countries

where the population and demand for food are greatest. Some of the negative consequences of climate change for ruminant livestock production can be mitigated using adaptive management and breeding approaches. Examples include the use of energy efficient cooling systems for animal welfare, proper feeding and access to water, the use of more heat tolerant breeds, and genetic selection to improve fertility and survival under heat stress conditions. Although it is limited, some improvement in resilience to recent climate extremes through adaptive management practices was achieved as documented by some livestock producers. Therefore, it is possible to put forward that improvements through feeding arrangements and management measures are relatively rapid (Henry et al., 2018).

In regions where climate change is not a problem, pasture-based livestock farming can be considered as a solution to eliminate potential negative effects. For example, it is suggested that pasture-based cattle and sheep farming systems in the UK could be climate neutral by 2030 and help restore biodiversity and soil health. A new consensus is needed that focuses on warming from emissions rather than emissions themselves, evoking more farmers to produce nutritious, affordable, quality food while trapping carbon, restoring nature and helping create rural economic resilience (Costain, 2019).

Reisinger and Clark (2017) carried out a study using previous estimates (1970-2010) and prospective projections of livestock emissions to find out some of the actual warming ascribable to direct livestock emissions except CO₂ today and in the future, and CO₂ from pasture conversions through a simple carbon cycle-climate model. In this study, it was determined that approximately 19% of the total modeled warming of 0.81°C from all anthropogenic sources in 2010 was caused by direct livestock emissions other than CO₂. CO₂ from pasture conversions contributed at least an additional 0.03°C, bringing the warming directly attributable to livestock to 23% of the total warming in 2010. The importance of direct livestock emissions in future warming is largely dependent on future global action carried out to reduce emissions from other sectors. If emissions from other sectors increase unabated, livestock emissions outside CO₂ will contribute only 5% of the warming in 2100. However, if global CO₂ emissions from other sectors are reduced to near or below zero by 2100 in accordance with the goal of keeping warming well below 2°C, they could account for 18% (0.27°C) of warming in 2100.

CONCLUSIONS

Worldwide extreme weather events in recent years and studies have made it necessary to investigate the effects of global warming induced global climate change on agricultural production systems more carefully and to produce immediate solutions.

It should be kept in mind that besides the action to be taken to prevent global warming should be primarily in the field of energy and industry, there are also some measures required to consider in livestock production. In order to meet the animal protein requirement of the increasing population, production systems that will increase efficiency in livestock production and reduce greenhouse gas emissions per unit production should be expanded. It is required that creating and using accurate models to predict the effects of climate change, performing sensitivity analyzes to determine the most suitable options for farmers, expanding studies based on interdisciplinary cooperation at local, national, regional and international levels in order to find-out miscellaneous solutions to climate change in livestock production.

With reference to the fact that animal production constitutes an integrity with plant production, according to the outputs obtained from climate models, the planting dates of forage crops should be adapted according to the new situation, the indoor conditions of livestock barn should be adjusted to provide animal comfort with some technical and managerial measures, and the studies on the development of breeds with high adaptation to new climatic conditions should be increased. Measures to increase the water holding capacity of the soil should be emphasized, and appropriate support and policy tools should be established in order to achieve all these goals.

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Chapter 6

Quality Maintenance In Seafood By Using Ozone

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ABSTRACT

Ozone as a residue-free cleaning agent has been used in the food industry for many years. The development of technology and the use of ozone as a safe cleaning and disinfection element is also important in the food industry, especially in ensuring quality and safety. Furthermore, the use of ozone, especially in perishable foods such as seafood, has enabled it to be a preferred technology application with the preservation of nutritional values. In addition, ozone is an environmentally friendly application and is a highly preferred processing method by the consumer. In this review study, researches such as quality changes induced by ozone application in seafood, preservation, the effect of different ozone doses on product quality were discussed and evaluated.

Keywords: ozone, seafood, quality, shelf life

INTRODUCTION

Aquaculture products are among the healthy foods due to their nutritional content and have been present in the human diet for many years, but they are in the category of perishable foods. In this context, they need protective measures from the moment they first leave the aquatic environment. In addition to edible films enriched with essential oils or preservatives, the use of technologies such as high pressure, radiation, superchilling, biopreservation alone or together have a very important place in the preservation of the quality of seafood (Pinto de Rezende et al., 2022; Jeon et al., 2002; Chéret et al., 2005; Hocaoglu et al., 2012; Mahto et al., 2015; Luan et al., 2017; Wu et al., 2014). Nonetheless, as stated by studies, ozone has long been used to sterilize food and water. (Gonçalves, 2019; Brodowska, 2017). However, although its use has been increasing in recent years, ozone application has been reported to be highly effective against bacteria, viruses and parasites (Kim, 1999; Ronholm et al., 2016; Silva et al., 1998; Liltved et al., 1995; Bartholomew et al., 2004). There are several benefits to employing ozone as a powerful oxidizing agent in food. It has the ability to reduce the counts of microorganisms and organic compound levels in a certain food matrix. Because of its strong oxidizing power and impulsively breakdown, ozone is also an effective disinfectant for guaranteeing the microbiological quality of seafood (Gonçalves, 2019; Kim et al., 1999). In recent years, the use of ozone in seafood has become a very popular topic, considering that consumers demand access to food with maintained quality, as well as the increasing consumer interest in foods that are processed in a way that is closest to their natural state and the demand for access to safe food. The present study investigated on the practical application of ozone in seafood, its effects on quality, application methods of ozone in seafood and the studies carried out in this context were compiled.

Quality changes affected by ozone in seafood

According to the studies, it is seen that ozone application is a considerably efficient technology for quality retention in seafood products. Bono and Badalucco (2012) investigated at the manner in which O₃ and MAP contributed to the quality maintenance of red mullet. (*Mullus surmuletus*). The researchers studied the quality changes in red mullet stored at +1°C for 21 days. The specimens were treated with ozone water (0.3 mg/l) and packed in a modified atmosphere consisting of 50% CO₂ and 50% N₂ gases. The researchers reported that O₃ treatment MAP were significantly effective in the microbiological quality of the samples and the number of microorganisms was significantly low until the 10th day of storage. In terms of chemical changes

(total volatile basic nitrogen (TVBN), trimethyl amine nitrogen (TMA-N) and peroxide value (PV)), they reported that these values were low in ozone-treated and modified atmosphere packaged samples and only in modified atmosphere packaged samples. In sensory terms, the researchers reported that appearance and odor parameters were maintained until the 10th day of storage. In another study, the microbiological, chemical and sensory changes of sardine (*Sardina pilchardus*) during storage in slurry ice, combination of ozone and slurry ice (SI) and flake ice (FI) were investigated during 22 days of storage. According to the results of sensory analysis, the shelf life of ozonated SI samples was reported as 19 days and the shelf life of the samples for SI and FI 15 and 8 days. Moreover, microorganism counts were reported to be lower in ozonated SI samples (muscle and skin). The researchers also reported that ozonated SI preserved the quality of samples during shelf life (Campos et al., 2005). In another study with ozonated SI, researchers examined microbiological, chemical and sensory changes in turbot (*Psetta maxima*) stored in ozonated SI for 35 days and at +2°C. In relation to the findings, nucleotide breakdown or TMA-N levels were not substantially impacted by the occurrence of ozone. However, lipid hydrolysis and oxidation were also reported to be slowed down. microbiologically, total aerobes and psychrotrophic bacteria were reported to be remarkably lower in samples for ozonated SI. researchers reported the shelf life of samples stored in ozonated SI as 14 days (Campos et al., 2006).

Ozone application in seafood is an effective method for the conservation of nutritional quality as well as the protection of microbial quality and prolongation of shelf life. Although the applied dose and application methods vary, studies investigating the effect of ozone applications on the quality and safety of seafood products are shown in Table 1.

Table 1: Ozone applications in different seafood products

Seafood	Processing and storage conditions	Ozone treatment	Quality parameters*	Effects of ozone	Reference
large yellow croaker (<i>Pseudosciaena crocea</i>)	FI, SI and ozonated SI, stored at +4°C	O ₃ water 1.8 mg/L, Ozonated SI 1.0 mg/L	TVC, PBC, H ₂ S producing bacteria and <i>Pseudomonas</i> sp, pH, TVB-N, TBA, BAs K-value, color difference and TPA	PBC, H ₂ S-producing bacteria, <i>Pseudomonas</i> , K value and BAs was lowered in ozonated slurry ice samples on day 21.	Lan et al. (2023)
black sea bream (<i>Sparus macrocephalus</i>)	Tea polyphenol combined with O ₃ water. packed samples were stored at +4°C for 15 days.	Tea polyphenol 1 dip 0.2% and O ₃ treatment 1mg/L	pH, K value, PV, TBA, TVB-N, TMA, texture, and colour, sensory analysis and TVC	Tea polyphenol and ozone combination reported to be effective in biochemical and microbiological quality, and maintained better characteristics of sensory quality compared with the control	Feng et al. (2012)
Cod (<i>Merluccius merluccius</i>), red shrimp (<i>Aristeus antennatus</i>), scald fish (<i>Arnoglossus laterna</i>) and musky octopus (<i>Eledone moschata</i>)	stored at +2°C for 12 days.	Passive Refrigeration-PRST TM thermobox with O ₃ generator system (500 mg/h)	TMAB and TPAB, Enterobacteriaceae, H ₂ S-producing bacteria, <i>Pseudomonas</i> , <i>Aeromonas</i> , <i>Brochothrix</i> spp. and <i>P. phosphoreum</i> . TVB-N and TMA-N, TBARs and sensory analysis	Extended shelf life by effecting the microbial growth. It is advised by the authors that the treatments should adjusted in terms of the fish species.	Aponte et al. (2018)
Atlantic salmon fillets	Stored in trays covered with bags at +4°C for 10 days	aqueous ozone at different concentration (1.0 and 1.5 mg/L), with different passess from the nozzles and contact surfaces	APC and <i>L. innocua</i> , TBARS, color analysis	It is recommended to employ O ₃ sprays at concentrations of up to 1.5 mg/L, which is effective in decreasing initial counts of APC and minimizing <i>L. innocua</i> counts without substantially elevating lipid oxidation rates.	Crowe et al. (2012)

*TVC: Total Viable Count, APC: aerobic plate counts, PBC: psychrophilic bacteria count, TVB-N: total volatile basic nitrogen, TMA: trimethylamine TBA: thiobarbituric acid, BAs: Biogenic amines, TPA: texture profile analysis, PV: peroxide value, TBARS: thiobarbituric acid reactive substance

Chen et al. (2016) evaluated the effect of O₃ and SI combination on shelf life and microbiological, biochemical and sensory quality changes in bighead yellow croaker (*Collichthys niveatus*). It was reported that total viable count, sensory changes, hardness and springerness values for ozonated SI in samples stored at +2°C for a total of 21 days differed significantly compared to samples stored only in SI and FI. In terms of shelf life, it was reported as 18 days for samples stored in ozonated SI, 15 and 9 days for samples stored in SI and FI. In addition, the researchers also observed the changes in myofibrillar proteins and reported that ozonated slurry ice delayed the decomposition of myofibrillar proteins and reduced microstructure degradation. Chen et al. (2014) aimed to determine the microbiological and chemical changes and shelf life of shucked oysters (*Crassostrea plicatula*) treated with ozonated water. In the study in which the effect of ozone application on quality changes and shelf life of shucked oysters was investigated, the samples were stored at +4°C. Regarding to the results of the study, it was reported that the total aerobic plate count value decreased approximately 2 log cfu/g in shucked oysters treated with 9mg/l concentration ozone for 10 minutes at 5°C. The researchers also observed that while O₃ treatment had no influence on TVB-N, TBA, or color values (a and b), it had a substantial effect on L values. Furthermore, the researchers observed that shucked oysters treated with ozone had a shelf life of 20 to 25 days.

CONCLUSION

As in the whole food industry, consumers want to have access to quality and safe food in aquaculture. As chemicals are used to maintain quality and safety in perishable foods such as seafood, negative results may occur at the point of preferability by consumers. Ozone application in aquaculture products is both effective and its use is increasing day by day due to the fact that it has no discernible effect on the nutritive and sensory characteristics of seafood products. However, because of the high oxidation properties of ozone, the inactivation and elimination of microorganisms in contact with perishable foods such as seafood, as well as the reduction of enzyme activity is an important advantage in maintaining the quality. Nevertheless, the high oxidation properties of ozone can cause protein degradation in seafood and in some cases, quality losses in color and texture properties. Considering the studies carried out in this context, it was concluded that ozone should be applied specifically according to the species, depending on the type of seafood, microbial load and storage conditions.

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Chapter 7

Use of Essential Oil Nanoemulsions Against Fish Pathogens

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ABSTRACT

Chemical applications in aquaculture are widely used for prophylactic and treatment in preventing fish diseases caused by infectious agents in intensive conditions. However, resistance development occurs with antimicrobial applications, and beneficial bacterial flora is eliminated with disinfectant applications. Therefore, undesirable environmental effects in aquaculture have emerged. Medicinal plants and their extracts and essential oils are used in the field of pharmacology as drugs or general health-supporting products. Medicinal plants have an increasing importance in their applications in aquaculture because of their biodegradable properties, they do not leave residues in the tissues and are safer for consumers. Today, the effectiveness of natural herbal products with nanotechnology is increasing and some difficulties related to the application are solved. Nanotechnology, which has found application in the pharmacology industry to develop more successful and reliable application methods, has also become possible to be used in new areas (agriculture, food, cosmetics, paint, perfumery, and chemical industries) in recently. Nanoemulsion practices, one of the new methods provide products resistance to physical conditions, prolonge their useful life by preventing decompose reactions such as evaporation, oxidation, and dehydration, increase the immobilization of microorganisms, increase fluidity, dispersibility, and solubility. Although there are some studies on the use of essential oils of medicinal plants against pathogens in aquaculture, it is crucial to develop new methods that increase the antimicrobial effect and be used in the sector.

Keywords: Nanoemulsion, fish pathogen, medicinal plant, aquaculture

INTRODUCTION

Aquaculture plays a crucial role due to its high protein content and significance in maintaining a healthy and balanced diet. Fisheries contribute 17% of animal protein consumption and 6.5% of the overall protein sources. The production of fishery products for food purposes has witnessed an average annual increase of 9.2% worldwide, with fishing accounting for approximately 90 million tons and aquaculture contributing around 87 million tons. However, alongside the industry's growth and production expansion, certain factors within the cultural conditions have given rise to problems such as infectious diseases. These diseases have led to substantial economic losses due to various reasons, including density issues, manual intervention, sudden fluctuations in temperature, poor water quality, and stressful conditions that compromise the immune system (Okmen et al., 2012:2762). Although preventive measures such as chemotherapy, vaccines, antibiotics, disinfection, and hygienic protection have shown partial success over the past two decades, the aquaculture sector continues to suffer from significant economic losses caused by diseases. In China alone, the annual economic loss due to aquaculture diseases amounts to \$400 million, while India and Thailand face losses of \$17.6 million and over \$500 million, respectively (Maqsood et al., 2011:147; Reverter et al., 2014:50; Cabello, 2006:1137; Naylor et al., 2000:1017; Quesada et al., 2013:1321).

Microorganisms, including bacteria, fungi, viruses, and protozoa, are causative agents for numerous infectious diseases in aquaculture. Antimicrobial agents have been employed against microorganisms. However, the use of these components has been limited due to their adverse effects on human health and environmental safety. To ensure the development and sustainability of the aquaculture sector, it is crucial to adopt an integrated approach that considers both living organisms and the environment. Extensive research has focused on exploring alternative methods for maintaining the health and productivity of aquatic organisms while minimizing environmental impact and reducing reliance on synthetic drugs and chemical substances. Herbal products offer a potential solution for promoting the sustainability of aquaculture by providing effective treatment options with minimal adverse effects on the environment. The use of herbal products in the treatment regimens for cultivated species holds significant importance in the realm of organic aquaculture. By embracing these natural alternatives, the aquaculture industry can contribute to organic practices and enhance overall environmental and ecological balance (Nik Mohamad Nek Rahimi et al., 2022:469).

Nanoscience encompasses the fusion of art and science, focusing on the manipulation of matter at the nanoscale to generate novel materials (Bektaş et al.,

2018:85). Within the realm of nanotechnology, nanoemulsions represent a significant methodology. These nanoemulsions can be described as conventional emulsions consisting of remarkably tiny droplets. Nanoemulsions are emulsion systems with a transparent appearance and droplet sizes varying between 20-200 nm (İlyasoğlu and El, 2010:143).

It has been demonstrated by research that essential oils of medicinal and aromatic plants have antimicrobial effects against various pathogens. However, it is known that essential oils are sensitive to changes in oxygen, light, humidity, and temperature and deteriorate over time (Gholipourkanani et al., 2019:871). In addition, since essential oils obtained from medicinal plants cannot mix in water, there are application difficulties. Due to their low surface tension, the materials brought into nanoemulsion form have very good wetting, spreading, and water-dissolving properties on the surface. Large surface areas also increase the absorption of the active substance. In addition, due to the small size of the droplets, the active substances they carry can reach the lower layers of the cell. It has also been stated that the materials brought into nanoemulsion form selectively combine with the cell wall or the lipid layer of the organism, and as a result, microbial cells (pathogens) are broken down (Solans et al., 2005:102).

Importance of medicinal and aromatic plants

Throughout history, humans have long acknowledged the therapeutic potential of plants and taken advantage of their benefits to attain health. In recent years, there has been a notable surge in the utilization of traditional medicine. Traditional medicine comprises a significant portion of health services in China, accounting for approximately 40% of all treatments. Similarly, in Chile, 71% of the population, and in Colombia, 40% of the population, have reported resorting to traditional medicine methods (Faydaoğlu and Sürücüoğlu, 2011:52). Additionally, a substantial proportion of India's rural population, specifically 65%, relies on traditional medicine practices to satisfy their fundamental healthcare needs. These statistics highlight the enduring prevalence and widespread adoption of traditional medicine practices worldwide.

Despite remarkable advancements in modern medicine and the pharmaceutical industry, alternative treatment methods and the use of medicinal plants continue to maintain their relevance. In recent years, there has been a growing interest in these practices, even in developed countries. Germany, for instance, stands at the head of this movement, with the public expenditure on traditional treatment methods amounting to 10 billion euros annually. Furthermore, it is worth noting that a significant portion of the global population, approximately 2.5 billion individuals residing in underdeveloped and developing

nations, particularly those with limited financial resources, are unable to access modern medicines. Consequently, they turn to alternative solutions such as Traditional Chinese Medicine (TCM), Ayurveda, Tibetan medicine, Unani medicine, acupuncture, shamanic medicine, and herbal treatments. Some countries incorporate these alternative methods into medical education and permit licensed physicians to practice them. The World Health Organization (WHO) recognizes the use of medicinal plants as a cost-effective alternative to pharmaceutical drugs. Additionally, promoting the use of medicinal plants allows countries to develop health technologies that align with their cultural heritage and natural resources, reducing dependence on developed nations (WHO, 2013:15). This perspective emphasizes the economic benefits and cultural compatibility of integrating medicinal plants into healthcare practices.

Essential oils of medicinal and aromatic plants

Plants are remarkable sources of natural products, including primary and secondary metabolites, which play vital roles in various industries, either directly or indirectly. Through their metabolic processes, plants transform water, minerals, and soil elements into compounds that can be assimilated by the human body. Primary metabolites, such as carbohydrates, proteins, fats, vitamins, and minerals, are essential nutrients required for basic bodily functions. In contrast, secondary metabolites contain a diverse array of active substances predominantly found in plant metabolism. Examples of secondary metabolites include etheric oils (essential oils, essences), alkaloids, terpenoids, saponins, glycosides, flavonoids, phenols, steroids, tannins, and bitter substances. These secondary metabolites often serve important roles in defense mechanisms and interactions with the environment. Essential oils, specifically, are mixtures derived from various plant parts (leaves, flowers, bark, seeds, and roots) through the process of distillation. They possess unique taste, color, and appearance, and exhibit volatile properties while remaining in a liquid state at room temperature. Although they are referred to as oils due to their insolubility in water and solubility in organic solvents, it is important to note that they differ from fixed oils (Bayaz, 2014:45).

Essential oils are characterized by their compositions, which can be classified into two main components: terpenes/terpenoids and aromatic/aliphatic compounds. Among these, terpenes hold significant importance and value in herbal products. Terpenes can be further classified into various types, including monoterpene, diterpene, sesquiterpene, hemiterpene, triterpene, and tetraterpenes. Aromatic compounds, specifically phenylpropanoids, are less prevalent compared to terpenes. While plants employ different biosynthetic

pathways for terpene synthesis, phenylpropanes are by-products of these pathways. Interestingly, both terpenes and phenylpropanoids contribute jointly to the antimicrobial activities exhibited by these structures (Çelik and Çelik, 2007:1; Faleiro, 2011:1143). Numerous scientific studies have highlighted the potent antibacterial effects of various secondary metabolites against both Gram-positive and Gram-negative bacterial strains. These secondary metabolites can serve as natural antibiotics, offering a safe alternative. Unlike synthetic antibiotics, which typically contain a single active substance, herbal antibiotics comprise numerous bioactive compounds. For instance, while penicillin just carries penicillin as its active ingredient, garlic, a powerful antibiotic, contains multiple effective substances, including allicin. Additionally, secondary metabolites possess antioxidant properties, capable of scavenging free radicals (Toroğlu and Çenet, 2006:12).

Nanoemulsion technology

Nanoscience is a multidisciplinary field that contains the design and engineering of functional systems at the molecular level. It serves as an applied science, focusing on the synthesis, characterization, and application of materials and devices on the nanoscale. Within the area of nanotechnology, nanoemulsions play a significant role and find wide-ranging use in industries such as food, cosmetics, and notably, the pharmaceutical industry. The pharmaceutical sector has witnessed a surge in the application of emulsions, particularly with the development of microemulsion and nanoemulsion formulations (Altav et al., 2020:149).

Fundamentally, the benefits of nanotechnology in drug delivery;

- Enhanced stability
- Visible resolution
- Increase in drug concentration (increase in efficacy)
- Decreased drug concentration in healthy non-target tissues (reduced systemic toxicity)
- Controlled release.

Emulsions are heterogeneous systems that exhibit a visually homogeneous appearance. They are formed by the dispersion of at least two immiscible liquids, typically a polar and a non-polar phase. In order for the emulsion, an oil phase, a water phase, and an emulsifier are required. The most important factor in the development of a successful emulsion formulation is the selection of an appropriate emulsifier (Çelebi et al., 2009:277; Çomoğlu and Gönül, 1997:95).

In addition to traditional emulsions, two new emulsion concepts, "micro and nanoemulsion", have emerged in recent years. Microemulsions; are clear, thermodynamically stable, isotropic liquid mixtures. They are prepared using oil, water, surfactant, and co-surfactant. They contain very small droplets compared to conventional emulsions. Nanoemulsions are very similar to microemulsions in terms of the distribution of nanoscale droplets, but unlike self-forming microemulsions, they are produced by applying mechanical force. In addition, they differ from microemulsions only in that they are kinetically stable (Çomoğlu and Gönül, 1997:95; Shah et al., 2010:24; Kumar et al., 2014:1031).

In many studies, the advantages of nanoemulsions are summarized as follows. The small size of the droplets enables them to remain suspended within the system. Additionally, their low surface tension imparts excellent wetting and spreading properties when they come into contact with surfaces. The significant increase in surface area facilitates enhanced absorption of the active substances. Unlike microemulsions, nanoemulsions require a much lower amount of surfactant (5-10%) for their formulation. The small droplet size allows for deeper penetration of the active ingredients into the lower layers of the skin when applied topically. Furthermore, nanoemulsions improve the water solubility of active substances with poor water solubility (Baboota et al., 2007:315; Mahajan et al., 2014:148).

Nanoemulsion forms of essential oils

In the field of pharmacology, nanoemulsion systems are employed to enhance the antimicrobial activities of essential oils and extracts derived from medicinal plants (Solans et al., 2005:102). Nanoemulsion systems consist of stable colloidal structures with droplets ranging in size from 5 to 100 nm. These systems offer several advantageous properties such as high physical stability, improved bioavailability, and low turbidity, which have contributed to their widespread utilization in the food, cosmetic, and pharmaceutical industries (Mou et al., 2008:270). The reduction in droplet size to the nanometer scale results in increased bioavailability and enhanced diffusion. Consequently, essential oil-based nanoemulsions exhibit improved efficacy as they can easily penetrate the sites where microorganisms proliferate. Furthermore, the combination of essential oils with surfactants and emulsifiers possessing hydrophilic and hydrophobic properties enhances the antimicrobial activity of their secondary metabolites (Ferreira et al., 2010:1383).

Several studies have demonstrated the antimicrobial effects of nanoemulsions on various pathogens in aquaculture. Valentim et al. (2018:1041) examined the antiparasitic properties of *Pterodon emarginatus* essential oil nanoemulsion

against a monogenean parasite (*Colossoma macropomum*). They observed a 100% anthelmintic effect when the nanoemulsion was applied at different concentrations (100, 200, 400, and 600 mg L⁻¹). In another study by Gholipourkanani et al. (2019:871), the *in vitro* antibacterial effects of nano-encapsulated herbal essential oils were investigated against different fish pathogens. The results indicated that the nanoemulsion formulation enhanced the effectiveness of essential oils, particularly *Origanum vulgare* nanoemulsion, as evidenced by lower MIC and MBC values.

Bedoya-Serna et al. (2018:929) investigated the antifungal effect of *O. vulgare* essential oil nanoemulsions against *Cladosporium* sp., *Fusarium* sp., and *Penicillium* sp. and found that it was effective against fungal species. Liang et al. (2012:7548) determined the effects of black pepper essential oil in nanoemulsion form against *Listeria monocytogenes*. They concluded that nanoemulsion technology increases the shelf life of food products. Donsi et al. (2011:1908) found that increased encapsulated terpenes and limonene nanoemulsions against *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, *Escherichia coli* species to the antimicrobial activity. El-Ekiaby (2019:13) determined that basil oil nanoemulsions have an *in vitro* antifungal effect with a MIC value of 50 µl ml⁻¹ against *Saprolegnia parasitica* fungal species that cause infections in Nile tilapia fish (*Oreochromis niloticus*).

Nazıroğlu et al. (2022:495) determined the antibacterial effect of the essential oils of two different aromatic plants (*Origanum onites*, *Mentha spicata* subs. *tomentosa*) and their nanoemulsion formulations against six different fish pathogens *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Aeromonas veronii*, *Yersinia ruckeri*, *Lactococcus garvieae*. The results of the study demonstrated that its essential oil nanoemulsions exhibited greater effectiveness against the tested pathogens compared to its essential oils alone. Another study focused on evaluating the chemical composition of essential oils from thyme (*Coridothymus capitatus*), laurel (*Laurus nobilis*), lavender (*Lavandula officinalis*), cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum zeylanicum*), and eucalyptus (*Eucalyptus globulus*) against various strains of *S. parasitica*. Additionally, the antifungal effects of nanoemulsions derived from these essential oils were investigated. Among the essential oil nanoemulsions tested against the fungal strains, only thyme (with MIC values ranging from 50 to 100 µl ml⁻¹ and MLC values ranging from 100 to 125 µl ml⁻¹) and cinnamon (with MIC values ranging from 250 to 500 µl ml⁻¹ and MLC values ranging from 500 to 1000 µl ml⁻¹) species demonstrated antifungal effects (Özil et al., 2022:2201). These findings highlight the potential of essential oil nanoemulsions, particularly those derived from thyme and cinnamon, as effective antifungal agents against *S. parasitica*.

CONCLUSION

Chemical applications have been extensively used in aquaculture for preventing and treating fish diseases caused by infectious agents in intensive farming conditions. However, the excessive use of chemicals in treatment has raised concerns regarding the accumulation of residues in fish meat, environmental pollution, and potential carcinogenic effects. In the field of pharmacology, medicinal plants, and their derived essential oils and extracts are gaining importance as drugs or products that promote general health. The increasing significance of the antimicrobial effects and biodegradable properties of medicinal plants in aquaculture applications stems from their ability to avoid residue formation in living organisms, ensuring consumer safety and environmentally sustainable production practices.

Nevertheless, the application of essential oils from medicinal plants in aquaculture encounters challenges due to their limited solubility in water. However, the development of nanoemulsion forms of essential oils has been reported to enhance their antimicrobial activities. This can be attributed to several factors, including the reduction in droplet sizes, which allows the nanoemulsions to remain suspended in the system, and their ability to spread effectively on contact surfaces due to their low surface tension. Furthermore, the improved water solubility of essential oil nanoemulsions contributes to their enhanced antimicrobial efficacy. These advancements in nanoemulsion technology hold promise for overcoming the application difficulties associated with the use of essential oils from medicinal plants in aquaculture.

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Chapter 8

The Use of Algae in Removal of Heavy Metal Ions from Wastewater

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ABSTRACT

1. INTRODUCTION

Technological developments offer many new and alternative products for the benefit of humanity. These developments also create different chemical wastes with organic, inorganic and radioactive properties at a considerable rate. Heavy metals such as Hg, As, Pb, Cr, Ni, Cu and Fe in wastewater; In addition to being poisonous and dangerous for aquatic organisms, it is dangerous for human health as part of the food chain (Gundogdu et al., 2016; Gundogdu et al., 2020). For this reason, the heavy metal content should be reduced before the wastewater is released into the environment and it should be ensured that it is reduced below the permissible values according to the water quality standards (Özer & Özer, 1998). Many methods are used in the treatment of wastewater, including physical, chemical and biological. However, in recent years, the application of biosorption methods has gained importance as it is economical, easily applicable and has no negative impact on the environment. Algae are one of the species used in the application of these methods (Becker, 1994). Algae, the primary producers of organic matter and part of the food chain, are used more and more in toxicity studies day by day due to the importance of their presence in the aquatic environment (Rainbow & Phillips, 1993; Sheata et al., 1999). Another use of algae is water filtration applications to remove nutrients, organic pollutants and heavy metals from wastewater (Torres et al., 2008; Karaduman et al., 2018; Karaduman et al., 2022). Algae can absorb metals dissolved in water both actively and passively, so the metal concentration in their cells may be several times the value in water. The disadvantages of using these organisms are that factors such as temperature and light intensity change the growth rates of living things and contamination occurs with dust particles adjacent to the surface of the algae (Taylan & Böke Özkoç, 2007). For this reason, the absorption and adsorption mechanisms of heavy metals in water should be well known. In addition, the advantages and disadvantages of the organisms used in metal removal and the biosorption method should be evaluated. On the side, the type of organism and method that is suitable for the characteristics of the wastewater and the most efficient result should be selected.

2. Absorption of Toxic Substances

Absorption is the phenomenon of substances passing through body membranes at a certain concentration. The passage of small-sized substances through the cell membrane occurs in two ways as passive and active transport, depending on whether the cell uses energy (ATP: Adenozin trifosfat). Passive transport is the passage of a molecule from a highly concentrated medium to a

less dense one using its own kinetic energy. Passive transport occurs in two forms, diffusion and osmosis. Substances to be transported through the cell membrane pass through the gaps between the fat and protein molecules in the membrane structure according to the diffusion rules. Diffusion; It is the passage of matter from the region where it is most concentrated to the region where it is less dense. It is a passive event. ATP is not consumed. Occurs in all living and non-living cells. Factors such as ambient temperature and molecular size are effective in transport. Passive transport occurs in living and non-living structures. Facilitated Diffusion; It is the passage of small molecules from a very dense medium to a less dense medium through the pores with the help of carrier proteins. ATP is not consumed. Osmosis; It is the diffusion of water through the semipermeable membrane. The phenomenon where the most dense part absorbs water from the less dense part is called. In other words, the dense part exerts a suction force on the less dense part. This suction force is called osmotic pressure. No ATP is consumed in osmosis (Vural, 2005; Utku, 2014; Demir, 2016).

In living cells, small molecules are also transported into the cell by active transport. Active transport is the name given to the transport of substance molecules from the environment where the density is low to the environment where the density is high, by expending energy in living cells. During active transport, enzymes and carrier proteins in the cell membrane work. In active transport, the substance to be transported binds to a protein carrier on one side of the membrane. With the energy provided by ATP, there is a change in the shape of the carrier protein. In this way, the substance that crosses the membrane is transported from a less dense medium to a more dense medium (Ritschel, 1994; Utku, 2014; Demir, 2016; Avcı, 2018).

Substance transport can occur from inside the cell to the outside of the cell or from outside the cell into the cell. An active cell must carry out active transport. Cells transport large molecules that cannot pass through the membrane according to diffusion rules, into the cell by endocytosis, by expending energy. The transport of large molecules out of the cell takes place through exocytosis by expending energy. Endocytosis: It is the event that large molecules that cannot pass through the pores in the cell membrane are taken into the cell by forming a pocket. This formed pocket deepens and breaks off from the membrane, and the membrane surface becomes smaller. During this time, energy is consumed. Depending on whether the ingested substance is solid or liquid, endocytosis occurs in two ways. Phagocytosis is the ingestion of large solids into cells. The process of taking large-molecule fluids outside the cell

membrane into the cell is called pinocytosis (Vural, 2005; Utku, 2014; Demir, 2016).

In order for toxic substances to have an effect in the organism, they must first exceed a certain concentration and pass through the body membranes at a certain concentration. The rate of entry of toxic substances into the body completely depends on the characteristics of the toxic substances and the organism's own absorption rate. The passage of substances across membranes occurs in three ways: active transport facilitated diffusion and endocytosis. In active transport, there is no concentration difference requirement and the substance is transported into the membrane with the help of trasportors. Toxic substance molecules cross the cell membrane by forming complexes with special carriers, and the complex substances collide and decompose, then come back again. Most secretory cells excrete their vacuole products by exocytosis. These secretions may be waste materials or substances produced by cells. The event that macromolecules that cannot pass through the cell membrane are thrown out of the cell is called exocytosis. Meanwhile, energy is provided from ATP (Vural, 2005; Utku, 2014; Murat, 2015; Demir, 2016).

3. Adsorption Mechanism of Heavy Metals

Adsorption or adhesion is a chemical and physical force related to the adhesion of atoms of dissolved solids and liquid and gaseous substances to the surface. Adsorption takes place in two ways, physical and chemical. Physical adsorption: it is the adsorption phenomenon that occurs as a result of the attractive forces between the particles, which can be in the form of atoms, molecules or ions, and the solid surface. Weak Van der Waals forces are at work here. Chemical adsorption: a chemical bond is formed between the particles and the surface, and these bonds occur either as covalent bonds or ionic bonds. Ions are attached to the charged areas on the surface with the effect of electrostatic attraction forces. Here, ionic strengths are important, and if the ions are equicharged, the smaller ones are preferentially held to the surface. In most adsorption events, physical and chemical bonds occur together or sequentially (Gupta & Keegan, 1997; Demir & Yalçın, 2014).

There are some factors that affect the adsorption process. These include solution pH, temperature, surface area, metal type and number (concentration). The pH of the medium plays an important role in adsorption. Since hydronium and hydroxyl ions are strongly adsorbed, the adsorption of other ions is affected by solution pH. In addition, the degree of ionization of acidic or basic compounds also affects adsorption. If the pH of the solution is high, the solubility of the metal complexes decreases and precipitate formation increases.

Due to the high concentration of H^+ ions at low pH, more H^+ ions will adhere to the cell surface rather than metal cations (Bozanta & Ökmen, 2011).

The adsorption process usually takes place in the form of an exothermic reaction (exothermic). Therefore, the adsorption size increases with decreasing temperature. It is known that the heat released is generally in the order of physical adsorption, condensation or crystallization heats, and in chemical adsorption, it is in the order of chemical reaction heat. The amount of adsorbed substance is determined as a function of concentration at constant temperature and the resulting function is called 'adsorption isotherm' (İleri et al., 1993). It has been reported that surface adsorption is a physicochemical phenomenon and is important in metal bonding efficiency. Since adsorption is a surface treatment, the size of adsorption is proportional to the specific surface area. Small particle size, large surface area and porous structure of the adsorbent increase adsorption (Vijayaraghavan & Yun, 2008).

A synergistic and antagonistic effect is observed in the sorption efficiency of metals, depending on the type and number of metals in the environment and the unique properties of these metals. In other words, a metal can affect the accumulation of another metal in a synergistic and antagonistic manner. For example, it is known that Cd and Zn have an antagonistic effect on some species or that Ni^{+2} and Cu^{+2} have a synergistic effect on the membrane transport of metals (Ting et al., 1991; Volesky et al., 1993). Studies on the biosorption of metals have shown that biosorption follows adsorption isotherms (Yakubu & Dudeney, 1986; Kuyucak & Volesky, 1988; İleri et al., 1993).

4. Heavy Metal Removal Methods

Many effective and economical new separation methods have been developed for heavy metal removal from wastewater. It is possible to examine these techniques in two main classes as abiotic and biotic methods. As abiotic methods, precipitation (chemical precipitation), adsorption, ion exchange, reverse osmosis, evaporation, membrane filtration and electrochemical technology methods can be shown. Since abiotic systems are technologies that are not environmentally friendly and generally depend on the concentration of the waste, they are not considered suitable for the removal of metals, especially in aquatic ecosystems, due to their high energy requirement and high cost. In recent years, commercial-scale biological methods have been intensified for the treatment of wastes (Vijayaraghavan & Yun, 2008). Applications using biological molecules for metal removal include biosorption, adsorption and phytoremediation methods. The biosorption method is more preferred due to its advantages. One of the advantages of biological methods is that they are

environmental, do not cause secondary pollution and are among the effective methods. Apart from these, low cost, high efficiency, no need for additional nutrients, regeneration of biosorbent, possibility of metal recovery are also seen as advantages (Kratochvil & Volesky, 1998; Bozanta & Ökmen, 2011; Hamutoğlu et al., 2012).

5. Heavy Metals and Biosorption Method

Biosorption is the process of removing waste materials from the aquatic environment by accumulating the biomass on or inside the cell surface (Sternberg & Dorn, 2002). It is a process based on making use of the Van der Waals forces and metal binding capacity of different biological materials. Biosorption of metal ions generally includes adsorption, ion exchange, complexing and microprecipitation, and is a rapid and reversible process (Kuyucak & Volesky, 1988; İleri et al., 1993; Hussein et al., 2004).

In heavy metal removal studies, the metal uptake capacity of dead or living cells was compared, and it was observed that the dead microorganism had a higher adsorption capacity in most cases (Aslan et al, 2007). It has been explained that the reason for this is that no inhibitory metabolic events occur during the transport of pollutants to the cell, the permeability of the dead cell membrane increases, and the cell surface properties change following the death of the microorganism (Stanley & Ogden, 2003; Demiroğlu, 2010). In the case of living organisms, some heavy metal ions can enter the cell by passing through the cell membrane. This form of metal capture is called intracellular capture or active capture. The situation in which both active and passive retention occur together is called bioaccumulation. Therefore, there is passive retention in metal uptake by dead cells, and both passive and active retention in metal uptake by living cells. In order for the biosorption process to be economical, it is aimed to use waste biomass instead of producing biomass (Shumate & Strandberg, 1985).

Microbial biosorbents are pre-treated to increase their sorption capacity of metal ions. These processes are carried out with different chemicals or at different processing steps. In addition to heat treatment, the use of organic chemicals such as formaldehyde, detergent and inorganic chemicals (NaOH, H₂SO₄, HCl, HNO₃, NaHCO₃, CaCl₂) are pre-treatments that kill microorganism cells, and then biosorbents are obtained by drying and sieving. It was determined that the biosorption capacity of dried and granulated sorbents increased significantly (Tzezos & Volesky, 1981; Vieira & Volesky, 2000).

Biological molecules used in metal biosorption show a wide distribution. In biological treatment processes, besides some plant and macroalgae species, various bacteria, yeast, fungi, plant species and algae species can be counted, especially within the microorganism group (Valesky, 1986; Sağlam & Cihangir, 1995; Table 1-2).

Dönmez et al. (1999) reported that many living organism groups (algae, bacteria, cyanobacteria and fungi) and particles obtained from these organisms are used to remove heavy metals from the environment.

Table 1. Organisms used in metal biosorption.

	Organisms	Metals	Resources
1.	Bakteria Species <i>Enterobacter aerogenes</i> <i>Pseudomonas aeruginosa</i> <i>Citrobacter</i> sp. <i>Arthrobacter</i> sp.	Cd, Ni Cd, U Th	Aksu and Kutsal, (1991) Boyd, (1970) Chigbo et al., (1979) Scott and Karonjkar, (1992) Standberg et al., (1981) Macaskie and Dean, (1984)
2.	Yeast Species <i>Saccharomyces cerevisiae</i> <i>Sporobolomyces roseus</i> <i>Kluyveromyces fragilis</i> <i>Candida utilis</i>	Zn, Cu, Cd, U Co, Ag	Huang et al., (1990) Han et al., (1992) Volesky et al., (1993)
3.	Fungi Species <i>Neurospora crassa</i> <i>Pleurotus ostreatus</i> <i>Rhizopus arrhizus</i> <i>Penicillium spinulosum</i> <i>Aspergillus niger</i> <i>Phanerochaete chrysosporium</i> .	Ni, Cd, Cu, U Au, Cd, Pb, Cu	Day et al., (2001) Ceribasi and Yetis, (2001) Kapoor and Viraraghavan, (1997) Shvaran et al., (1992) Favero et al., (1991) Sağlam et al., (1994) Zhou and Kiff, (1991)
4.	Plant Türleri <i>Azolla pinnata</i> <i>Eichharnia crassipes</i> <i>Lycopersican esculentum</i> <i>Nicotiana tobaccum</i>	Pb, Zn Cd, Pb, Hg	Boyd, (1970) Jain et al., (1990)

Table 2. Algal species used in metal biosorption.

Algal Species	Metals	Resources
1. Microalgae		
<i>Chlorella homosphaera</i> <i>Chlorella vulgaris</i> <i>Aseophyllum nodosum</i> <i>Scenedesmus carinatus</i> <i>Sargassum natans</i> <i>Scenedesmus acutus</i> <i>Spirulina platensis</i>	Zn, Co, Pb, Cd, Hg, Zn, Fe, Au, As Cu, Cr	Travieso et al., (1999) Sudharto et al., (2014) Aksu and Kutsal (1991) Ting et al., (1991) Chigbo et al., (1979) Brinza et al., (2007) Michalak et al., (2007) Tajes-Martinez et al., (2006) Rai and Mallick, (1992)
2. Macroalgae		
<i>Ascophyllum nodosum</i> <i>Lessonia flavicans</i> <i>Lessonia nigresense</i> <i>Laminaria japonica</i> <i>Laminaria hyperbola</i> <i>Ecklonia maxima</i> <i>Ecklonia radiata</i> <i>Durvillaea potatorum</i> <i>Chaetomorpha Linum</i> <i>Sargassum</i> sp. <i>Padina</i> sp. <i>Ulva</i> sp. <i>Gracillaria</i> sp. <i>Chaetomorpha Linum</i> <i>Caulerpa lentillifera</i> <i>Ulva Lactuca</i> <i>Cladophora fascicularis</i> <i>C. Linum</i>	Cu, Pb,Cd, Zn, Ni	Yu et al., (1999) Sheng, et al., (2004) Hashima and Chub, (2004) Pavasant et al., (2006) Lodeiro, 2006. Murphy et al., (2007) Michalak, et al., (2007) Brinza et al., (2007) El-Sikaily et al., (2007) Deng et al., (2007) Ajjabi and Chouba, (2009)

6. Types of Algae Used in Metal Absorption

Algae, one of the important living communities of the aquatic ecosystem, provide the oxygen of the aquatic environment as well as creating an organic food source for heterotrophic creatures by photosynthesis. Algae without real roots, stems and leaves, according to their size; They are divided into microalgae and macroalgae.

-Microalgae: They are living things that do not have movement organelles, can be replaced by water movements, and whose dimensions are expressed in microns. Microalgae are microorganisms that contain carbohydrates, proteins, lipids and vitamins. In general, depending on the species, microalgae can contain about 15-77 % oil (Xu & Miao, 2006; Chisti, 2007; Ulukardeşler & Ulusoy, 2012). Abdel-Aty et al. (2013) stated that the use of microalgae as biosorbent is more promising. As a reason, they stated that microalgae can be cultured more easily than macroalgae, they have high production efficiency, the efficiency of the biosorption area up to micron size and their high performance.

De Filippis and Pallaghy (1994) explained that microalgae are found in very large quantities in natural environments, from fresh waters to seas, and have an important ability to remove heavy metals (Trollope et al., 1976; Şen et al., 2003; Çölkesen Doğru, 2010).

-Macroalgae: They are vegetative organisms that vary in size from 1-2 cm to 40-50 m, depending on the species, and form the feeding, sheltering and breeding environment for aquatic creatures. According to the pigments they contain, they are divided into three classes as Brown (Phaeophyceae), Red (Rhodophyceae) and Green (Chlorophyceae). Macroalgae, one of the multicellular eukaryotic organisms, is one of the important living resources of the seas (Ak, 2015). Algae are found in very large masses in fresh waters and seas. The abundance and widespread availability of algae in the coastal areas of the world, being a renewable natural biomass, low cost, high metal absorption capacity and regular availability have greatly increased their usability as biosorbent (Apiratikul & Pavasant, 2008). Metal biosorption studies using macroalgae mass are quite numerous. The macroalgae *Sargassum* sp. *Padina* sp. (brown), *Ulva* sp. (green) and *Gracillaria* sp. (red) species were investigated for biosorption performances in the removal of Pb, Cu, Cd, Zn and Ni metals from aqueous solutions. It has been determined that the metal uptake capacity of algae species varies depending on the pH values, and the metal uptake capacity is higher at low pH. It was noted that Pb, Cu and Cd metals were taken at the highest level compared to the other two metal (Zn, Ni) ions. In general, brown algae *Padina* sp. and *Sargassum* sp. demonstrated the highest potential as biosorbents for the removal of heavy metals from aqueous solutions (Sheng et al., 2004).

7. Absorption Mechanisms of Algae

The sorption of metals by algae is affected by many factors such as the algae used, cell size and cell wall composition, morphology and physiology, pH, temperature, metal specificity, metal concentration and biomass density (Mehta & Gaur, 2005; Güçlü & Ertan, 2011). The absorption mechanisms of microorganisms differ according to the species. The sorption mechanisms occur in the form of extracellular accumulation (in solution), attachment on the cell surface and accumulation in the cell (Sud et al., 2008; Figure 1).

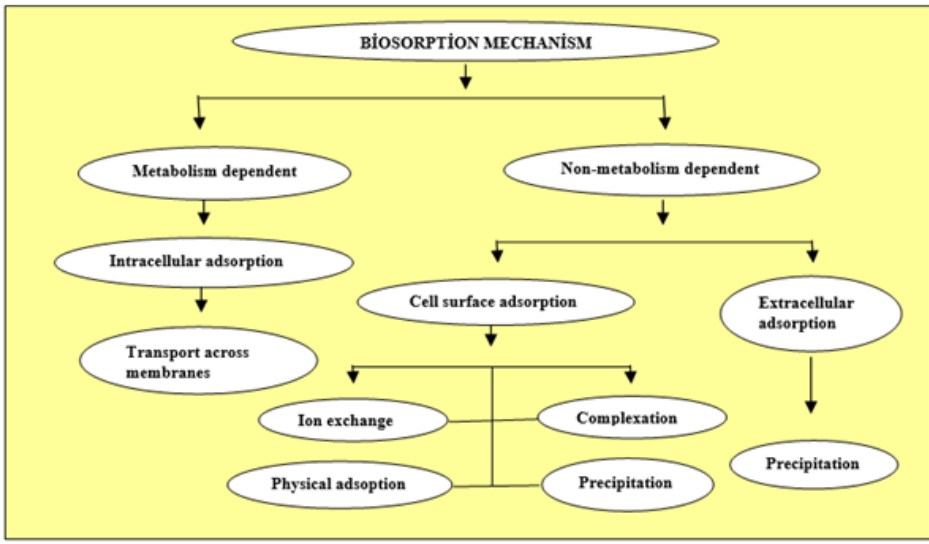


Figure 1. Absorption mechanisms of microorganisms (Güçlü & Ertan, 2011)

Sorption in Cell Metabolism: Metal sorption kinetics with organism cells occurs in two steps. The first step is physical adsorption or ion exchange on the surface of the organism. This step is often called passive removal. This step is very rapid and equilibrium is established shortly after the organism interacts with the metal. Rapid removal is usually the result of surface adsorption (Ting et al., 1989; Brady et al., 1994). Adsorption to the solid surface is usually due to the high affinity of the solid surface. Affinity depends on physical, chemical and ionic strengths (Akkaya, 2005). The second step in metal uptake is the slower, intracellular removal step due to metabolic activity, which includes the transport of metal ions across the cell membrane. This step is called active removal (Ting et al., 1989). Metabolism-dependent functioning is an efficient transport process with generally slower uptake and is irreversible (Veglio & Beolchini, 1997; Mehta & Gaur, 2005). It involves many mechanisms, including adsorption, diffusion into the cell, ionic and covalent binding, redox reactions, binding to proteins and other cellular sites. This process begins with the transport of metal ions to the cytoplasm and the cell membrane forming the outer boundary. When metals enter the cell, they can bind to their intracellular components or precipitates. Detoxification occurs by the accumulation and binding of metals to the intracellular proteins in the vacuoles of the algae. Metals in algae accumulate especially in lipid-rich tissues and intracellular structures (Güçlü & Ertan, 2011). Metal accumulation occurs in the form of binding to cytoplasmic organelles, metallothioneins, phytochelatins and other

intracellular molecules, apart from the metal being retained on the cell surface (Mehta & Gaur, 2005). It has been explained by many researchers that some organisms can synthesize polymers extending from the outer membranes of their cells, and that these polymers have the ability to bind metal ions from solution (Tsezos & Volesky 1981; Norberg & Persson, 1984).

Physical adsorption, ion exchange and complex formation occur in the physicochemical interaction between the functional groups (polysaccharide, proteins, organic acid and peptidoglycan) on the cell surface and the metal. This is called sorption on the cell surface. In the algae cell wall, there are many functional groups such as (-OH), phosphoryl ($-\text{PO}_3\text{O}_2$), ($-\text{NH}_2$), ($-\text{COOH}$), sulfhydryl ($-\text{SH}$), $-\text{SO}_4^{2-}$, which give a negative charge to the cell surface. Since metal ions in water are usually cationic, they can be attached to the cell surface (Gadd & De Rome, 1988; Stevenson et al., 1996; Veglio & Beolcini, 1997; Bozanta & Ökmen, 2011). Proteins in the cell wall form active regions to bind metal. Heavy metals have a strong affinity for proteins. The nitrogen and oxygen of the peptide bonds of proteins, groups such as hydroxyl, amino, and phosphate can be replaced by metal ions (Tsezos & Volesky, 1981). Ampholite proteins have a certain isoelectric pH depending on the type of molecule. It is stated that if the positively charged metal ions are below the isoelectric point, the groups contained in the protein molecules are replaced by the same charged ions with cationic character. On the other hand, it is thought that at pHs above the isoelectric point, they are adsorbed by forming complexes with negatively charged reaction sites (Sağ & Kutsal, 1995). Therefore, it can be interpreted that the pH of the environment is an effective parameter in heavy metal adsorption.

Since the distribution and abundance of cell wall components vary according to algae groups, the number and type of functional groups also vary according to algae groups. Among the different cell wall components, polysaccharides and proteins are the most important of the metal binding sites. The carboxyl terminus of cell wall polysaccharides is highly functional in heavy metal absorption by algae. It was stated that the $-\text{SO}_4^{2-}$, $-\text{NH}_2$ and $-\text{OH}^-$ groups participated in the absorption of different metal ions, but the participation was lower than the $-\text{COOH}$ groups (Mehta & Gaur, 2005).

Cell Sorption Mechanisms: In organism cells, sorption occurs in four steps. These are physical attachment, transport into the cell membrane, complex formation and precipitation.

-Physical Attachment: The phenomenon in this absorption mechanism is associated with Van der Waals forces. Weak van der Waals attraction was observed between the retained material and the retaining surface. Kuyucak

& Volesky (1988) argue that there is electrostatic attraction between the cell wall and the ions in the solution in the absorption of U, Cd, Zn, Cu and Co by dead algae, fungi and yeast biomass.

-Transport into Cell Membrane: This type of absorption takes place only with living cells and is related to cell metabolism. Transport of heavy metals into the microbial cell membrane; It is the same mechanism used in the transport of metabolically essential ions such as K, Mg, Ca and Na (Veglio & Beolchini, 1997).

-Ion Exchange: Ion exchange is a reversible chemical reaction (Figure 2). Generally, the ion exchange mechanism is illustrated by the following equation.



(HY= Number of acid points on solid surface; Mx^+ = Metal ion; MY_x = Absorbed Mx^+)

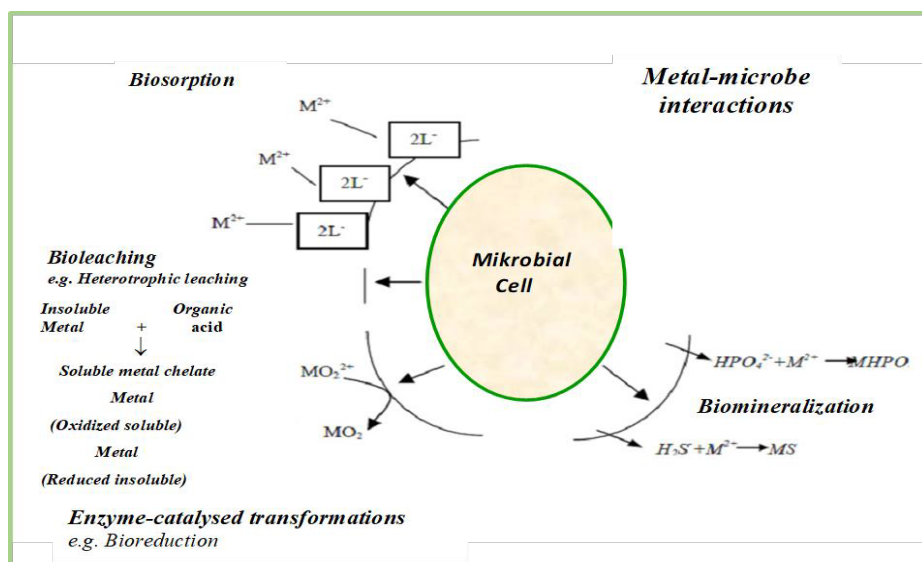


Figure 2. Metal–microbe interaction mechanisms that can be harnessed for bioremediation applications (Lloyd et al., 2002).

The main building blocks of the cell wall of microorganisms are polysaccharides. The details of the ion exchange properties of the polysaccharides were investigated and it was determined that the +2 valence metal ions and the ions of the polysaccharides corresponding to these ions were replaced. The marine algae biome usually contains natural salts K^+ , Na^+ , Ca^{+2} and Mg^{+2} . It is stated that these metallic ions are replaced by corresponding ions

such as Cd^{+2} , Co^{+2} , Ni^{+2} , Cu^{+2} and Zn^{+2} and the absorption of metals takes place (Veglio & Beolchini, 1997). The ion exchange capacity is highly variable according to the algae species. The diversity of ion exchange capacity in algae species is due to the difference in cell wall composition. Due to the high surface-to-volume ratio of unicellular algae, the ion exchange capacity is generally greater than that of filamentous algae. As a result, the ion exchange capacity and metal uptake capacity are proportional to the pH increase in the algal bioma (Mehta & Gaur, 2005).

-Complex Formation: Metal removal from solution also occurs with complex formations on the cell surface as a result of the interaction between metal and active groups. Metal ions can also bind with chelate formation (Cabral, 1992). Ionic strength is also an effective factor on biosorption. For example, some inorganic ions such as chlorine affect the biosorption process by forming complexes with some metal ions (Vijayaraghavan & Yun, (2008). On the surfaces of some microorganisms, high molecular weight polyphosphates or chemically similar groups bind the metal to themselves in the form of complexes. For example, *Citrobacter* sp. Phosphatase enzyme, which releases inorganic phosphate from the organic phosphate in the cells, allows the heavy metal to precipitate as the cell-bound metal PO_4^{-3} (Macaskie & Dean, 1987; Utku, 2014).

-Precipitation: Precipitation can be both dependent on cell metabolism and independent of metabolism. In the first case, metal removal from the solution occurs by active defense systems of microorganisms. In the presence of toxic metals, they react and precipitate compounds are produced. Where precipitation is not dependent on cellular metabolism, chemical interactions occur between the metal and the cell surface (Veglio & Beolchini, 1997).

8. Advantages and Disadvantages of Biosorption Method

-Advantages: Dead biomass is usually easily obtained from waste or a natural source. The cost is low, the efficiency is high. Since biomass is non-living, reproductive parameters can be eliminated. Metal adsorption is fast and highly efficient. The biosorbent material generally acts as an ion exchanger. They are not affected by metal toxicity like living cells. The metal is easy to desorption or can be recovered. The biosorbent is reused (Demiroğlu, 2010). The system can be described mathematically. Depending on the microorganism activity, metabolic products are not synthesized. Chemical or biological sludge can be reduced. Dead cells can be stored for a long time at room temperature.

-Disadvantages: The cell surface becomes saturated with metal very quickly. When the places holding the metal on the surface are filled, it is

necessary to desorb the metal for further purification. Dead cells do not have the potential to biologically alter the valency of the metal, which facilitates precipitation. Adsorption is sensitive to effects such as pH. Adsorption of some metals takes place at very low pH. Cu^{+2} , Ni^{+2} and Pb^{+2} occur in the pH 4-5 range. Organic species do not have the potential to break down metabolically.

The disadvantages of using living organisms in biosorption are the need for additional nutrients. Factors such as temperature and light intensity are effective in the growth of living things. Contamination with dust particles adjacent to the surface of the algae can occur (Taylan & Böke Özkoç, 2007), and many microorganisms can grow at low pH.

9. Conclusion

Heavy metals, which are directly toxic to the aquatic environment and organisms, are usually accumulated at significant levels by aquatic organisms. Considering the food chain, this accumulation is likely to have harmful effects on both aquatic organisms and human health. Therefore, direct mixing of heavy metals into water resources is very dangerous and alarming. Parallel to the developing technology, the importance of treatment systems and methods is increasing as a result of the increase in the heavy metal content of wastewater day by day. In order to increase efficiency in wastewater treatment with living organisms, microorganisms that can be obtained from industrial wastes or nature and that reproduce rapidly should be selected. In many studies conducted today, macroalgae and microalgae species are used as organisms in the biosorption system. Necessary information should be provided on the advantages of using algae species, especially as biosorbent in biological treatment systems, and research on this subject should be supported.

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Chapter 9

Organic Poultry in The World and in Türkiye

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Abstract

Today, emerging diseases and the deterioration of the ecological balance have led consumers to prefer products that do not harm human health and are produced with environmentally friendly production models. In addition, the organic poultry sector has started to attract attention with people's desire to buy animal protein and the thoughts of least harming the environment. Organic poultry farming can be defined as a breeding system in which animal rights and welfare are taken into consideration more and animals are housed in environments suitable for their nature as much as possible, considering the different taste perceptions and sensitivities of consumers. In this production model, economic priorities and sensitivities to the growth performance of animals are not at the forefront. There are significant differences between them in terms of production performance, cost, chicken breeds used, rules and food characteristics when compared to conventional or industrial chicken production. Both organic chicken meat and chicken meat are valuable foods for people, and consumption preferences should not be guided by false and false information. Chicken meat is an important, delicious, healthy and cheap food for our people's right to adequate nutrition. Organic chicken meat is also of the same quality, but its price is quite expensive due to the production model, which is the most important disadvantage for the consumer.

Key words: Organic, poultry, Türkiye

1.INTRODUCTION

Poultry farming provides an affordable source of nutrients and has made significant contributions to the world's food security. Despite recent advancements in efficiency, there is still a biosecurity gap because of infectious diseases and rising feed prices (Alves et alç 2023, Khan et al 2023, Kadawarage 2022) Organic livestock, on the other hand, is a type of breeding that has not been genetically modified thanks to natural seeding, is resistant to environmental climatic conditions and diseases, is fed with completely organic feeds, is suitable for recreational areas (such as open field and pasture) and living conditions. implemented in accordance with current conditions and applicable legislation. (Karaarslan and Parıldar 2013, Gerbens et al 2013, Vaarst 2015).

Livestock is an important sector of organic agriculture. Poultry meat and eggs are the cheapest animal protein sources, and it is estimated that 50% of the world meat production will be met by poultry species in 2050. Poultry production is the first thing that comes to mind as a solution to the problem of increasing food demand due to increasing population growth. The biggest reasons for this situation are the cheap and abundant products obtained as a result of studies in the field of genetic improvement and feed technology (Karaduman and Nariç 2022) . Organic livestock is an environmentally friendly production method that allows farm animals to show all their natural behaviors, is fed with organic feeds, does not use additives such as hormones and antibiotics to increase efficiency, and offers healthier products to consumers. Cutting off the relationship between animal husbandry and soil and plant production is against the nature of the animals, and at the same time, it causes problems in meeting animal feed from reliable sources. In addition, the animal manure produced also causes environmental pollution to a large extent.

For these reasons, there are problems in organic farming without including organic livestock. Organic production is a mixed production system that deals with plant and animal production together. Organic production has emerged in agriculture mostly in plant production branches and has started to become widespread. However, in developed countries, the organic animal husbandry era has begun in agriculture, as consumers prefer animal food products with high nutritional safety, as is the case with plant products, and with the increase in environmental awareness and sensitivity to animal rights. In the demand of organic products by consumers; The importance they attach to personal health, and especially to the health of children, is in the first place. In a survey study conducted to determine the reasons for preference of organic products in EU member countries, it was seen that health came first. After that, environmental

awareness, product taste and sensitivity to animal rights come respectively. (Karaarslan and Parıldar 2013).

Organic livestock methods provide a system that provides appropriate environment and animal welfare in terms of animal rights, protection of ecological balance, sustainable livestock, fighting against animal diseases and protecting the health of herds. This situation contributes to the country's economy by ensuring the continuity of the quantity and quality of the products obtained from stress-free animals and increasing the income levels of the producers (Şahan et al. 2015). However, intensive breeding methods cause health problems in animals. In addition, pesticides used in agriculture, hormones used in animals, antibiotics and pesticides cause many negativities in human health. Organic agriculture is an important production method that should be applied to protect humanity and the environment (Karaarslan and Parıldar 2013).

In organic poultry, it is aimed to create an organic flock and to obtain healthy products from healthy animals by protecting the health of chickens with an organic breeding and feeding. The development of the chick embryo depends on the storage of nutrients in the egg. Feeding animals at high levels leads to increased concentrations of some nutrients in eggs (Ayaşan and Baylan 2011).

After all; It is expected that with the poultry products to be obtained according to the organic farming criteria, people will be fed with healthier and safer animal foods, and animals will be physiologically more comfortable with care and nutrition in more natural conditions compared to intensive production.

In this study, the situation of organic poultry in the world and in our country was examined by giving general information about organic livestock.

2. ORGANIC LIVESTOCK

2.1. Principles and Purposes

Many countries and many private certification bodies have defined organic agriculture. While there were differences in the definition of organic agriculture before, the search for a partnership in the definitions of all international trade organizations has created a unity in this regard. The International Federation of Organic Agriculture Movements (IFOAM), a non-governmental organization that supports organic agriculture internationally, creates generally accepted and applied guidelines on organic production and processing. The approved basic principles of organic agriculture are grouped under four headings.. (Rehber 2013a, IFOAM 2021).

Health principle: According to this principle, the health of the ecosystem cannot be considered separately from the health of people and society. Health means the integrity and intactness of living systems. It is not just the absence of

disease, but the maintenance of physical, mental, social and ecological well-being. Immunity, stamina and regeneration are key features of health. The role of organic farming is to maintain and improve the health of ecosystems and organisms, whether in farming, processing, distribution or consumption, from the smallest living things in the soil to humans. In particular, organic agriculture aims to produce high-quality, nutritious food that contributes to preventive health care and well-being. In this respect, the use of fertilizers, pesticides, animal pesticides and food additives that may have negative effects on health should be avoided.

Ecology principle: This principle is based on organic farming within living ecological systems. It explains that production will be based on ecological processes and recycling. For example, in the case of crops, this is living land; is the farm ecosystem for animals; It is the aquatic environment for fish and marine organisms. Organic management must be adapted to local conditions, ecology, culture and scale. Inputs must be reduced through reuse, recycling and efficient management of materials and energy to maintain and improve environmental quality and conserve resources. Organic farming must achieve ecological balance through designing farming systems, creating habitats, and maintaining genetic and agricultural diversity. Those who produce, process, trade or consume organic products must protect and benefit from the common environment, including landscapes, climate, habitats, biodiversity, air and water.

Principle of Fairness (Openness): Organic farming should be based on open relationships with respect to the general environment and living possibilities. This principle has features such as equality, respect, justice and protection of the shared world in both people and their relations with other living things. This principle establishes human relations between farmers, workers, industrialists and consumers in a way that is open and honest in all situations. Organic farming contributes to the well-being of all, food independence and poverty reduction. This principle envisages that animals should be looked after in appropriate conditions for their psychology, natural behavior and life. The principle of openness envisages the creation of an open, equitable, production, distribution and trade system that takes into account both environmental and social costs. (Rehber 2013b).

Principle of care: Organic Agriculture is a lively and dynamic system that responds to domestic and foreign demands and conditions. Organic farming practitioners can increase efficiency and productivity, but this should not risk compromising health and well-being. As a result, new technologies need to be evaluated and existing methods need to be reviewed. Given the lack of understanding of ecosystems and agriculture, care must be taken. This principle states that the most important issues in the management, development and

technology selection in organic agriculture are precautions and responsibility. It is essential to ensure that organic farming is healthy, safe and ecologically sound. However, scientific knowledge alone is not enough. Practical experience, accumulated wisdom, traditional and indigenous knowledge offer valid solutions tested by time.

2.2. Advantages

Consumers are turning to organic nutrition for reasons such as health, nutritional value, animal health and protection of their rights. One of the most important benefits of organic farming is that it protects the environment and causes minimal harm. We can list the benefits of organic animal husbandry as follows (Tayar 2013, Bayram et al. 2013).

1. It is important for human and animal health and welfare.
2. It supports herbal production. Crop production and animal production constitute both a circular economy and a circular ecological balance. Thus, it is ensured that agricultural pollution is prevented.
3. Since chemicals such as hormones, antibiotics and pesticides, which are used too much in traditional production, are not included in organic production, inputs are saved.
4. Diseases seen in traditional livestock herds are less common in organically grown herds.
5. Since animal welfare is prioritized, there is no decrease in stress hormones and related diseases in animals.
6. Antibiotic immunity is not observed in herds, as antibiotic feeds are not used in organic livestock farming.
7. By protecting small producers, it ensures the social balance of the society to survive.
8. It provides an additional capacity opportunity for the export of organic products, increasing the export volume.
9. Ensuring the follow-up of the producer and the products through certification is of great importance in terms of food safety

3. ORGANIC CHICKEN EGG FARMING

The main differences of organic egg production from traditional production are focused on animal management, feeding, medicine and treatment.

Hybrid chickens are generally used in organic egg production around the world. In some countries such as England and the Czech Republic, brown layer lines suitable for organic production maintenance-management conditions have been developed. In order to eliminate or reduce the problem of excessive hair

pulling and cannibalism in ecological free-range systems, genotypes with brown but white hair have been genetically developed. In some countries, it is more common to use local breeds that give dark brown eggs by small producers locally and are highly preferred by consumers. Considering factors such as hybrid chickens being less resistant to diseases and environmental conditions, it can be said that local breeds or pure culture chickens are more suitable for organic production. However, some studies have shown that pure breeds are not efficient enough in organic production. According to the Regulation on the Principles and Implementation of Organic Agriculture of the Ministry of Food, Agriculture and Livestock (2010, Substances 16); in organic livestock; Breeds with high adaptability to environmental conditions and resistant to diseases should be selected, and priority should be given to indigenous breeds and crosses adapted to that region. (Petek 2013).

Table egg production is widely carried out in the cage system throughout the world. However, it is forbidden to use the cage system in organic production, and free-range housing systems, which generally consist of a closed shelter and a walking/grazing area, are used intensively. In the UK, the free circulation system is mostly used in the closed shelters with the grid-mat system, in the Netherlands the birdhouse system is widely used.

According to the regulation, the shelters should be constructed from a hygienic building material and should be in accordance with the biological and racial characteristics of the animals. Animals should have easy access to feed and water. Insulation, heating and ventilation system of buildings; The air flow inside the shelter, dust level, temperature, relative humidity and gas density should be kept within limits that will not harm the animals. The shelters should be large enough for the animals to move freely and naturally, lie down easily, turn around, clean themselves, and perform all natural movements such as stretching and flapping their wings. The minimum areas and shelter features of closed shelters and open walking areas must comply with the conditions specified in the regulation. At least 1/3 of the coop floor should be in a flat structure with a litter system and should be covered with materials such as straw-straw, sawdust, rice husk, short grass or sand. (Öztürk and Türkoğlu 2012, Petek 2013, Cessari et al 2017).

According to the regulation, more than 1/2 of the floor in egg coops should be suitable for collection of feces. Perches should be built in proportion to the flock group and chicken size. Depending on the size of the chickens, the entrance/exit holes in the grazing area should be at least 4 m long for each 100 m² of the poultry house (Regulation Clause 19). Minimum areas of closed shelters and outdoor recreation areas and other shelter features for different animal species and categories must comply with the criteria specified in the regulation. (Petek 2013).

The care and management of animals in organic production is generally the same as in traditional production. Chickens should be kept in open rearing conditions with free range. The use of the cage system in organic production is prohibited, and beak cutting should not be applied in this aquaculture.

The yield period in laying hens is a maximum of 16 hours a day with natural and artificial lighting. Hens should be allowed to rest by applying darkness for at least 8 hours a day. This is also necessary for ovulation to occur. Chickens should be able to easily reach the outdoor walking-grazing area as long as the climatic conditions are suitable. Strolling-grazing areas should be mostly covered with vegetation, have guards such as canopies, and be suitable for animals to reach an adequate number of drinkers and feeders.

Biosecurity measures to protect public and animal health should always be applied carefully, without interruption. The aim in organic production should be the production of safer and higher quality food, especially in terms of weed pesticides, fertilizers and other chemical additives, instead of obtaining the maximum product per unit area. Ecologically produced egg yolk attracts attention with its darker-natural color. In a study, it was stated that quality characteristics such as egg yolk color were better in organic eggs than eggs produced in cages. The food quality of ecological products also varies according to the conditions of classical production in the same region/country. (Petek 2013).

4. ORGANIC BROILER FARMING

Organic broiler production is lower than organic egg production. It has been determined that it is close to egg production in some European countries. There are fundamental differences in the areas of shelters, care methods, feeding methods, drug use and treatment used in organic production compared to traditional production.

In organic production, the use of modern hybrids used in traditional production is not recommended. Among the main reasons for this are factors such as the low ability of these animals to withstand environmental conditions and their unsuitability for animal welfare. It is recommended to use slow growing broiler genotypes instead of modern hybrids in ecological broiler breeding. Slow growing broiler slaughter age is approximately 12 weeks. During this period, there are weight gains of less than 45 grams per day. Thus, grazing, walking and perching behaviors are fully developed compared to fast-developing ones. Since they are more active, there is no need to make a feed restriction.

An ecological broiler production, consumer preferences are as important as the producer in the selection of breeds. For example, if consumers consume more breast meat, genotypes with more breast meat should be selected. In ecological

production, it is very important to select and develop genotypes that are resistant to diseases and environmental conditions.

A closed barn and a free-range barn system should be used in organic broiler breeding. Small businesses may also prefer closed portable shelters. While portable shelters provide more benefit from the pasture, they can increase labor costs. The total number of animals to be housed in the henhouse is defined in the regulation. Compared to traditional production, the number of animals is kept less in ecological production. The number of animals per unit area in pastures and open areas should be limited in such a way as to provide sufficient animal manure for crop production in the production section. Animal density should be determined in terms of the amount of nitrogen emitted, not exceeding 170kg/N/ha/year in the agricultural area used.

One of the most important differences of ecological production from traditional production is animal feeding. Chickens should be fed with balanced rations in terms of nutrients prepared from certified ecological feed raw materials produced according to ecological production rules. However, there are various difficulties in the production of ecological feed raw materials. Therefore, difficulties are encountered in creating a balanced feed component. In ecological production, forced feeding of animals should be prevented and animals should be fed with the feed specified in the regulation. Feedstuffs, feed additives, feed processing aids and animal feeding products; cannot be produced using genetically modified organisms or products derived from them. The regulation should be taken into account for the conditions sought in ecologically produced or processed feeds. It is of great importance that preventive medicine and biosafety rules are applied very carefully and sensitively in organic chicken farming. For the purpose of biosecurity, measures should be taken in accordance with biosecurity principles such as preventing the transmission of disease agents to the enterprise, killing disease agents, providing care and feeding in accordance with the age and physiological needs of animals, and establishing adequate immunity.

When starting ecological production, the area where the fodder crops are made and the animals are taken into the transition process. The transition product is marketed with the label "Organic agriculture transition product". Expressions that evoke organic agriculture should not be used in advertisements and labels of products obtained from animals in the transition period. In a farm or production unit that is partially engaged in ecological agriculture or is in the process of transition to ecological agriculture, the producer should keep the ecologically produced products in such a way as to distinguish between the products and the transitional products and animals, and must have records to show this distinction.

The land and pastures where ecological animal production will be made should be included in the two-year transition period. If the lands have not been treated with products other than those specified in the regulation, the transition period can be reduced to 6 months by authorized institutions. (Petek 2013, Anonim 2010).

5. CONTROL AND CERTIFICATION OF ORGANIC CHICKEN EGGS AND BROILER CHICKENS

Organic products are products with certain characteristics, different from other methods and grown according to certain rules. While organic product owners or sellers claim that their products are organic, buyers will have questions. This situation creates consequences such as perceiving organic products as normal products, misleading consumers and unfair competition. The most effective way to eliminate this distrust arising from the information difference between the buyer and the seller is certification, except for closed systems where the producer and the consumer act together (Rehber 2013a).

Any product that has not been produced in accordance with the provisions of the Regulation and does not have a "Product Certificate" indicating that it is an organic raw material and/or organic processed product cannot be marketed as an organic product. Every entrepreneur selling organic products must certify each batch of sales with a product certificate. These products are sold with the clear indication that they are organic. Organic products must carry one of the logos specified in the regulation in the domestic market (Anonim 2021).

All plant and animal products produced in accordance with the Organic Agriculture Law and Regulation are evaluated as organic and offered for sale with the label and organic product logo detailed in the regulation. (Anonim 2021).

Labels must clearly state that the product is an organic product. Organic product logo should be present (Figure 2.2). The name, logo, certificate number of the control and/or certification body and the code number given by the Organic Agriculture Committee must be present. The parcel number where the product was produced and the content of the product should be included in a complete list. The place of production, production and expiry date of the organic product should be specified. Colors to be used in logos (Figure 2.1); green, blue, black and white (Günşen 2013, Anonim 2010).



Figure 2.1. Colors to be used in logos



Figure 2.2. Examples of logos to be used in organic agricultural products

With the amendment made in the Turkish Food Codex Egg Communiqué in 2014 by the Ministry of Food, Agriculture and Livestock, a stamping requirement was introduced for "A class" eggs used directly by consumers or industries. In this case, the business and house number of the egg has started to be stamped on the egg shell. During sales and marketing, it has been determined that different expressions such as "organic eggs", "village eggs", "natural eggs", "free range chicken eggs" on egg labels mislead the consumer and cause unfair competition among producers. Thereupon, the ministry brought the obligation to stamp the "breeding method code" on the egg shell with the change in the communiqué on November 24, 2017. These codes are grouped under four headings and the numbers are given automatically by the system. The breeding method code is automatically given by the system in front of the holding and house number, as 0 for organic farming, 1 for free range aquaculture, 2 for cageless aquaculture and 3 for caged aquaculture. (Anonim 2017).

6. MARKETING IN ORGANIC LIVESTOCK

The demand for organic products in our country has started to increase since the early 2000s. Organic production was carried out for export purposes in the first years of implementation. Approximately 20% of the herbal production produced in our country is consumed within the enterprise itself, and 80% is offered to the market. The marketing share of animal products is not as high as in herbal products. 40% of animal products are offered in-house, and approximately 60% market share. (Can et al. 2013). The low market share of animal products compared to herbal products is primarily due to low production. In order for animal production to take place, there is a need for both organic plant production and large areas where animal welfare can be achieved. This situation also affects consumer demands as it is reflected in the prices of organic animal products. With the development of the market, sales started in the organic product markets opened in the big cities under the supervision of non-governmental organizations and with the support of the municipalities. Considering consumer demands, sales began to be made on special organic product shelves of supermarkets and boutique organic product markets.

Organic products; Since they do not undergo any processing during production, collection, packaging and distribution processes, they show less durability compared to products produced by traditional methods. This situation has led consumers to buy organic products in less quantity and as much as they can store. In this context, after the products are harvested, the logistics organization must be realized very quickly. The shorter the distribution process, the greater the success of the manufacturer in marketing its products. With the development of the distribution channels of organic products, there has been an increase in the product variety and it has become more profitable for the producer.

Costs and sales figures are important in organic production. Some of the main reasons for high product costs; The need for more human labor than traditional production, low efficiency, control and certification costs. In order for the products to bring more profit to the manufacturer, purchasing directly from the manufacturer is among the leading preferences, usually with a single agent or no intermediary (Külekçi and Aksoy 2015).

The reliability of organic products for consumers is protected by regulations and certification is provided by authorized organizations. In this way, the traceability from the seed to the plate has also become questionable by the consumer.

In the marketing of organic products, informing the consumer and the producer as well as the product, and carrying out training activities with regular announcements play an important role. In the marketing studies carried out with

the intermediary, the fact that the intermediaries also have knowledge about the products and organic farming processes increases the demand for the producer's product. (Vural and Turhan 2013).

7. ORGANIC POULTRY FARMING IN THE WORLD AND IN TURKEY

Organic animal husbandry has not developed as much as plant production in Turkey as in the world. As the level of consciousness and awareness of consumers increases, the demand for organic products also increases. In addition, developments in organic livestock breeding are accelerating with the thoughts of animal welfare, access to healthy food and respect for the environment. Among the livestock sectors, breeding and feeding methods in the poultry sector are among the fastest and most appropriate production models in the nutrition of societies. Egg and chicken meat are more preferred by consumers compared to red meat due to their economic and nutritional properties. This has accelerated the development of organic poultry farming.

In recent years, organic production and consumption have been increasing rapidly in the world. Turkey ranks first in the number of producers in Europe and among European Union countries. When we look at the years 2014-2015, there is a significant decrease in the number of animals, meat and milk in organic livestock, while an increase of 22.7% in the rate of organic eggs has been detected. (Çelikyürek and Karakuş 2018).

Table 1: Presence of Organic Laying Hens in The World (2012-2019)
(Piece)

Countries	2012	2013	2014	2015	2016	2017	2018	2019
Belgium	320194	277822	281657	362569	352046	665390	825850	905960
Greece	-	-	-	67765	-	-	-	-
Spain	124229	185247	211318	253102	313632	444796	629508	833937
France	335687	3351274	3734551	3894718	437201	502003	659283	773331
					3	4	4	3
Italy	201068	1901639	1904439	1865558	-	-	-	-
Cyprus	-	3550	.616	4187	8111	8162	10354	12680
Holland	212080	2089448	2285152	2574561	-	321089	362319	375564
						3	3	0
Austria	809790	-	625222	740625	831477	854826	875475	915800
Portugal	-	-	-	-	-	35949	48362	51766
Türkiye	-	187102	254611	362806	575180	657407	635380	749736

Source:https://ec.europa.eu/eurostat/databrowser/view/ORG_LSTSPEC/default/table

* Data could not be found in places with (-) signs in the table.

Organic laying hens and broiler chicken production in the world by years are given in Tables 1 and 2. When we compare it with developed countries, it is seen that Turkey's production volume remains low. While it is seen that organic egg production has increased over the years, we can say that organic broiler meat production is fluctuating. Particularly, the great decline in 2019 and more research and development on organic poultry are thought to be a great necessity for both producers and consumers and the country's economy.

Table 2: Presence of Organic Broiler Chickens in The World (2012-2019)

Countries	2012	2013	2014	2015	2016	2017	2018	2019
Belgium	1512538	1620430	1710047	1905657	2167775	2639764	3269147	3683472
Greece	-	-	-	210250	-	-	-	-
Spain	71806	151748	165696	195231	251170	362900	387457	968820
France	7879959	7956858	8572485	8639351	9728125	11372916	12921180	14165410
Italy	779046	1123420	1542255	2211102	-	-	-	-
Cyprus	-	6000	3000	3000	11035	11465	13976	8553
Holland	77740	82930	61626	77260	-	84509	99866	136197
Austria	339089	-	457868	597579	980699	1132177	1248722	1390986
Portugal	-	-	-	-	-	12.094	8.989	10.587
Türkiye	-	694512	834167	589804	608862	604900	606790	94583

Kaynak: [https://ec.europa.eu/eurostat/databrowser/view/ORG_LSTSPEC/default/table\(2020\)](https://ec.europa.eu/eurostat/databrowser/view/ORG_LSTSPEC/default/table(2020))

* Data could not be found in places with (-) signs in the table.

Organic agriculture has started to show its effect in the poultry sector in recent years. However, the organic carnivorous chicken and egg farming sector has not developed enough yet and is open to new investments. When we examine the amount of organic carnivorous chicken production in Turkey, while the total amount of meat was one thousand two hundred and sixty-one tons in 2018, this amount decreased to six hundred and ninety-seven tons in 2019. When the number of producers is examined, while there were twenty-six producers in 2018, this number decreased to six in 2019. While the number of chickens was 606 790 in 2018, this number decreased to 94 583 chickens in 2019. (Table3). It was observed that while some of the producers stopped producing organic poultry, some of them increased the number of organic laying hens instead of organic carnivorous chickens. (Anonim 2020, Öztürk et. al. 2013).

Table 3: Organic broiler chicken production in Türkiye (2018)

Provinces	Number of	Number of	Meat production
Afyonkarahisar	2	18000	40
Elazığ	1	21990	54
İzmir	7	20400	21
Sakarya	15	8800	18
Samsun	1	537600	1128
Toplam	26	606790	1261

Source: <https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Organik-Tarim/Istatistikler>, 2020

When we look at the amount of organic egg production in Turkey, while it was around ninety three million in 2016, it was about one hundred and sixty one million in 2017. It reached approximately one hundred and seventy-five million in 2018 and approximately one hundred and eighty million in 2019. The number of producers increased to twenty-seven in 2016, to seventy-seven in 2017, to one hundred in 2018 and to one hundred and five in 2019 (Table 4). Considering these data, there are serious increases in organic egg production and the number of enterprises in 2016-2017. While the number of provinces where producers are located was fourteen in 2016, it increased to twenty-one in 2019. When we look at the total egg production in Bursa, it is observed that while it was approximately 1.6 million in 2017, this number decreased to around one million in 2019. (Anonim 2020, Öztürk and Türkoğlu 2012).

Table 4: Organic egg production in Türkiye(2019)

Provinces	Number of plant	Number of chickens	Meat production (ton)SSayısıüretimi(adet)
Adana	1	9818	3000000
Afyonkarahisar	1	6000	1836000
Ankara	2	1065	65700
Bolu	9	106760	32016781
Bursa	2	11808	1042581
Çorum	1	9000	2000
Elazığ	1	43250	11000000
Erzurum	3	600	75000
Gaziantep	1	5500	
İzmir	6	80212	17000000
Kırklareli	4	26591	6449014
Kocaeli	2	2786	826672
Konya	4	16900	
Malatya	2	4300	1380000
Manisa	3	101551	25947082
Ordu	28	26400	7578000
Sakarya	15	150400	35964000
Samsun	3	97180	30149795
Tekirdağ	2	19206	426390
Trabzon	4	2139	216924
Uşak	11	28270	4805562
Toplam	105	749736	179781501

Sources:<https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Organik-Tarim/Istatistikler>, 2020

8. CONCLUSION

With the diseases that have emerged in recent years, the deterioration of the ecological balance and the climate crisis, consumers have started to prefer products that are produced with environmentally friendly production models that do not harm human health. However, the organic poultry sector has started to attract attention, with people wanting to buy animal protein and thinking of causing the least harm to the environment.

Although organic plant production has developed before organic livestock breeding, it should be carried out together with organic livestock production in order to increase organic plant production and progress in a healthy way. Because there is not only one living species in the ecosystem. Producers should be taught to use the systems together and even policies should be developed on these issues. Thinking only about feeding the society means dragging the society to hunger in the following years. Production plans should be made to feed the communities in a way that causes the least damage to the ecosystem.

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Chapter 10

A Review Of Function Of Freshwater Crayfish Gastroliths And Their Usage Areas

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ABSTRACT

INTRODUCTION

Considering the world population growth, crustaceans provide important contributions to both the economy and human health by meeting the food needs of people due to their rich protein content. Global aquaculture production of crustaceans reached 11.2 million tons in 2022 (FAO, 2022). Due to the edible rate (<50%) of crustaceans, a large amount of shell waste is also produced during meat processing, and green recycling of crustacean shells is gaining more and more attention (Sato et al., 2010). Crustaceans, with about 50,000 to 75,000 species, are an important group of arthropods. Shrimps, crabs, crayfish, lobsters, and other large crustacean species have an important place in aquaculture in terms of rich food sources. Crustaceans their derivatives are an important sustainable part of modern culture and commerce, biomedicine and economic development, aesthetics, gastronomy, geology and many other fields (Susanto, 2021; Amiri et al., 2022). Crustaceans survive by occupying benthic, pelagic, planktonic and intertidal niches. Most crustaceans have motile, sedentary and parasitic lifestyles, and these are arthropods that are more dominant in the oceans (Susanto, 2021).

Decapod crustaceans constitute a group of arthropods with approximately 15,000 species among more than 170 families (Crandall et al., 2009). Freshwater crayfish are represented by 737 species and subspecies in the world (Crandall, & De Grave, 2017) and approximately 15 of these species are of economic importance (Holdich, 1993; Mazlum, 2003). Although there are quite a lot of species, they are generally known as families (Parastacidae, Cambaridae, Astacidae) that are economically important for hunting and culturing purposes (Eversole et al., 2006) (Figure 1).



Figure 1. Species belonging to the family (a) Parastacidae), (b) Astacidae) and (c) Cambaridae (Original)

They feed on crayfish, other invertebrates, macrophytes, algae and detritus. They play an important role in the freshwater food chain. Crayfish also display a cannibalistic trait and have a selective diet that consumes certain invertebrates and macrophytes. When they are abundant, they contribute significantly to the food chain and species diversity, either as a direct consumer or as a consumable creature. In addition, crayfish are an important source of energy for predators (Mazlum, & Yılmaz, 2012).

Crayfish are invertebrates, taxonomically called arthropods, crustaceans or decapods (Mazlum, & Yılmaz, 2006). Crayfish are omnivorous and have the ability to adapt to the environment in terms of physiological, morphological and behavioral characteristics. Crayfish are very important aquatic organisms for the freshwater ecosystem, due to their key roles in the trophic niche, acting as modulator of food webs, and can be found abundantly and predominantly among all invertebrates (Laffitte et al., 2023). While some crayfish species prefer cold waters with plenty of oxygen such as lakes and rivers, other species can also live in warm water environments that are poor in dissolved oxygen. Some species are well adapted to living in brackish waters (Reynolds et al., 2013; Emery-Butcher et al., 2020). At the same time, crayfish are important food sources for animals living in many aquatic environments. The presence of crayfish in freshwater ecosystems has a significant impact on biodiversity (Wood et al., 2017).

Crayfish are rich in protein and an excellent source of minerals and vitamins compared to fish (zinc, iron and Vitamin B-12, choline, etc.). Crayfish meat is a good source of protein, contains 16-18% protein and is a low-calorie food. It is also rich in minerals such as sodium, potassium, magnesium (Mazlum, & Uzun, 2022; Zhang et al., 2023). Although crayfish are also used as bait in fishing, most of the cultivated species are used as human food. There are four stages of life cycles of crayfish: these are called eggs, hatchlings, juveniles, and adults (Figure 2). Freshwater crayfish have no larval stages and all development typical of free-living decapod larvae takes place inside the egg, so the hatched larvae is a young crayfish already having most of its normal adult appendages. When the eggs hatch, the young crayfish initially remain attached to the female. At this time young crayfish undergo three molts. Then young crayfish leave female and become free living. Young crayfish total length is about 10 mm during this time (Vogt, 2008).

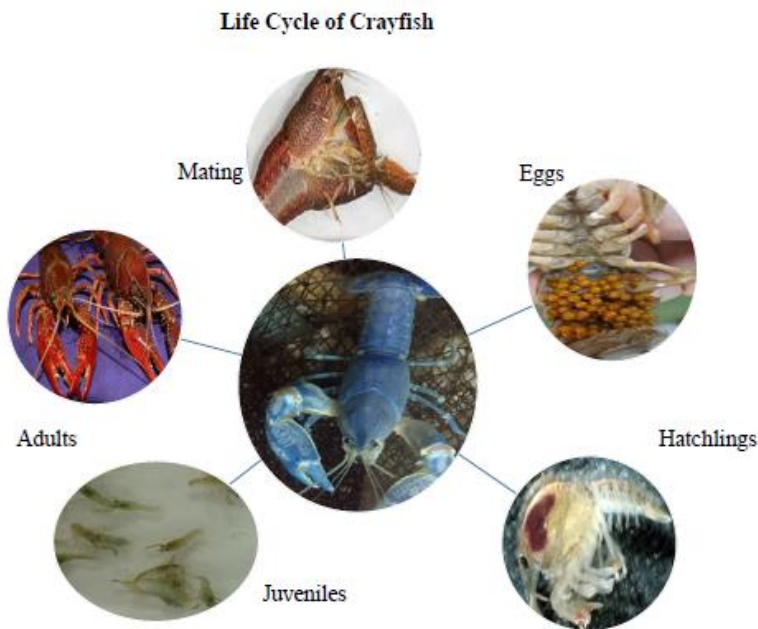


Figure 2. Life Cycle of Crayfish

Structure of crayfish exoskeletons

The structure of the exoskeleton of crayfish consists of a calcium-chitin mixture and protein (Blackwell, & Weih, 1980; Lowenstam, & Weiner, 1989). Raabe et al., 2005 reported that chitin-protein fibers of the exoskeleton form a helically stacked network of chitin-protein layers in a twisted plywood model. The rigidity of the exoskeleton occurs due to the enzymatic oxidation of phenols or catechols. Phenols or catechols then interact with cuticular proteins and chitin to cross-link and harden them in a process known as sclerotization (Kuballa, & Elizur, 2008). The exoskeleton is further hardened by the deposition of minerals, especially calcium carbonate, in most crustacean species (Lowenstam, & Weiner, 1989). In crayfish, the exoskeleton (shell) acts as a barrier protecting the living thing against harmful organisms (Luquet, 2012; Nagasawa, 2012). Therefore, crayfish are highly resistant to diseases. Protein-rich crustaceans are produced in millions of tons worldwide, and these valuable food sources contain inedible parts in the form of shells that make up more than half of their body mass. The shells of crustaceans contain chitin, the second largest natural polysaccharide after cellulose. Crayfish shells contain chitin, a long-chain polysaccharide with an antibacterial effect when applied as a plaster, helping to heal severe burns faster and prevent scarring. Also, chitin is used for nasal spray vaccines, thus eliminating the need for needles. It can also be considered as an alternative

method to fear of needles, which is the reason for not being vaccinated against Corona virus, which is an important disease in our country and in the world.

Biom mineralization

Crustaceans are an important group of animals notable for biomineralization, capable of developing a cyclically mineralized exoskeleton (Luquet, 2012; Shaked et al., 2020). Moreover, they can cyclically synthesize calcified structures not only in terms of their morphology, but also in terms of their mineralogical composition. Ecdysis then rapidly stores calcium ions in an amorphous form (Jamil et al., 2022). Why these amorphous mineralization structures are time stabilized compared to chemical minerals remains a mystery (Luquet, & Marin, 2004; Abehsera et al., 2021). Another most important feature of calcium metabolism in terms of transport in crustaceans is that it is very similar to vertebrates. Namely, the calcium pumps and enzymatic systems associated with the calcium transport epithelium in vertebrates are similar to the working system of crustacean tissues. It arises through hormonal regulation between two sources of calcium for crustaceans. This situation occurs in invertebrates with the triple vertebrate calcium control system (CT/PTH/vitamin D) (Weiner et al., 2021).

Molting of Crayfish

Most of the events taking place in the crayfish's body are controlled by compounds called hormones produced by the endocrine glands. Hormones are synthesized from a sinus gland located near the eyes and from certain nerves. These hormones, carried by the blood flow, each regulate a different activity. Some of these control color on the body surface so that the crayfish can modify its color. Some other enzymes regulate the reproductive cycle by stimulating the development of reproductive glands during appropriate seasons. Others, on the other hand, regulate molting by ensuring that calcium and other salts in the lower layers of the exoskeleton are removed or protected from the body (Mazlum, & Yilmaz, 2012). The main mechanism in the molting process in crayfish is the endocrine system. The endocrine system is affected by biotic and abiotic factors. The molting process results from the physiological changes that the hormones produced by the X-organ, together with the sinus gland and the Y-organ. The X-organ and sinus gland together inhibit the molting process. The Y-organ has the typical cell elements synthesizing steroids with abundant secretory vesicles and abundant mitochondria mainly in the pre-molt phase. It synthesizes the Y-organ ecdysone hormone and its derivatives. These hormones are steroids and they regulate the somatic growth and regeneration of individual body parts (Weiner et al., 2021).

Molting is the removal of the hard shell and the creation of a new one. As in all arthropods, the growth of crayfish is not continuous as in fish, but is based on a periodic increase in body length (Sandeman, & Sandeman, 2000; Mazlum, & Yilmaz, 2012; Abehsera et al., 2021) (Figure 3).

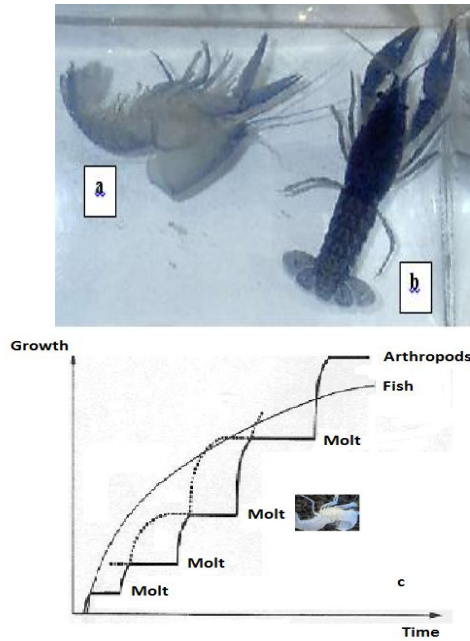


Figure 3. a) Adult specimen of freshwater crayfish
b) After molting crayfish c) Growth

In some crustaceans, molting occurs throughout the life cycle, while in other species molt takes place only until sexual maturity is reached (Hartnoll, 2001; Xiong et al., 2023). Exoskeleton change is very rare in crayfish that reach sexual maturity. In a short period of time, which includes the hardening of the new shell, the crayfish rapidly increases its mass by taking water and air into its body tissues. The animal then fills in the expanding new shell, forming new tissues. During this period, very rapid growth is achieved. In arthropods, tissue growth occurs in the intermolt phase. Growth is based on the proportional increase in body mass. After the shell hardens, there is no change in the external size of the crayfish. In the period after molting, the need for mineral substances, primarily calcium, is quite high. After molting, reserve substances are released from the hepatopancreas and gastroliths, which enable the recalcification of important body parts. Crayfish sometimes balance their mineral needs by eating their old exoskeleton. Remineralization means that the exoskeleton is first hardened with

chitin and then calcified. During the hardening of the exoskeleton, the crayfish continues to grow. Due to the rapid onset of food intake, body reserves are used for recalcification of the mouthparts. The recalcification of the body is completed in a very short time, 2-3 days. After this step, the crayfish begin to receive nutrients again. In the period between molts, crayfish have a fully developed calcified exoskeleton, take up nutrients in a standardized manner and produce spares for the next cycle (Kozak et al., 2015).

The molt cycle in crayfish generally consists of four stages; soft stage: this is a sensitive period for crayfish, during which the new skeleton develops (Freeman et al., 1987). During this period, crayfish have a soft shell. In this period, especially if the number of shelters is low and the population is dense, there are losses due to cannibalism and aquatic predators. Postmolt stage: it includes the hardening process of the skeleton. In this phase, the calcium used in the initial hardening of the shell is supplied from the body and water. During the feeding period of the crayfish, the mineral accumulation of the shell increases. Intermolt stage: this stage is defined by a fully formed and hard outer shell (Vega-Villasante et al., 2007). In crayfish, the shell consists of 4 layers. These are from the outside to the inside; it consists of epicuticle (upper shell), exocuticle (outer shell), endocuticle (inner shell) and membrane (membrane) (Roer, 1984, Simkiss, & Wilbur, 1989) (Figure 4). During this period, crayfish feed, increasing both their energy reserves and their tissue mass. This phase may last longer or shorter depending on tissue growth and accumulation of organic reserves, and this situation changes closely related to nutrition. Premolt stage: it includes the formation of new soft shell and the expulsion of the old shell. In the later stage of the premolt, the crayfish stop feeding and seek shelter (Freeman et al., 1987). Calcium is excreted from the exoskeleton and during this period the calcium content in the blood also increases to contribute to the structure of the skin soft shell. Molt phase: The shell change phase is the expulsion of the old shell, which is completed in a short period of time. At this stage, the crayfish increase their volume by taking in water. In the dorsal region, the exoskeleton is separated between the carapace and the abdomen and the shell is removed with the help of tail movements (Cabrera, & Griffen, 2023; Wærvågen et al., 2016; Su et al., 2021).

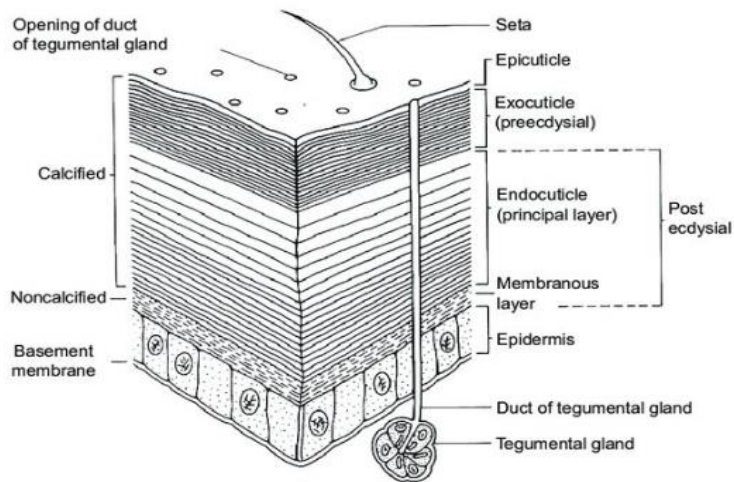


Figure 4. A section view of the crayfish exoskeleton (from Reynolds 2002).

Function of Gastroliths

The function of gastroliths (stomach stone) is still debated and several theories have been generated regarding them (Wings, 2007). The most frequently cited hypotheses are those that discuss hydrostatic function (buoyancy) (Taylor, 1993; Henderson, 2006) and their use in the digestive process. The most widely accepted hypothesis by researchers is that they are used in the digestion process (Cicimurri, & Everhart, 2001; Everhart, 2000; Cerda, & Salgado, 2008) (Table 1). Because stomach stones depend on the activation of the epidermis, they are excellent indicators of the molting stage (Travis, 1965; Vu et al., 2023). At the same time, gastrolith deposition is accepted as an indicator of molting in crayfish (Gorissen, & Sandeman, 2022).

Table 1. Percent composition of crayfish gastroliths (Scott, & Dunhan, 1967).

Ca ⁺⁺	%38
CO3	%53
PO4	%9.1
Mg ⁺⁺	Trace
SO4	Trace
Cl	Trace
HCl	%1

Stomach stones, like the exoskeleton, consist of a chitin protein organic matrix in which calcium carbonate accumulates (Luquet, & Marin, 2004) and epithelial formation is continuous (Nudds et al., 2022). Studies have shown that stomach

stones serve different purposes in crayfish and other crustaceans (Pavey, & Fielder, 1990; Greenaway, 1985; Wu et al., 2023). Crustaceans have a hard calcium carbonate exoskeleton, which is shed during molting (Abehsera et al., 2019). Once crayfish shed their shells their new shells are soft and thus helpless to predators, so crayfish need to build a new exoskeleton as soon as possible (Nhut et al., 2020). A process called "ecdysis" that causes the change of their shells, the hormones that trigger the shell change in crayfish trigger the removal of calcium carbonate from the exoskeleton. This causes the formation of a double stomach stone in the epithelium of the stomach wall (Shechter et al., 2008; Nhut et al., 2020). However, it has been suggested that the growth rates of gastroliths may differ between seasons, broad bands may be formed in gastric stones in faster growth and narrow bands in gastric stones in slower growths (Vu et al., 2023).

Once the crayfish has shed its entire shell, it begins to reabsorb calcium from the stomach stones in the stomach wall, where it stores calcium ions in the form of calcium carbonate to form new shells (Jamil et al., 2022). Moreover, crayfish eat the discarded shell, providing CaCO_3 for their new exoskeleton. It has been reported that the Ca^{+2} is very little in fresh water. Mud crabs, lobsters and crayfish have gastroliths called stomach stones (Luquet, & Marin, 2004). Stomach stones in crayfish and other crustaceans have been recognized as a calcium store, often used during calcification of the new exoskeleton (Robertson, 1941). Stomach stones in crayfish and other crustaceans have been recognized as a calcium store, often used during calcification of the new exoskeleton (Robertson, 1941).

Gastroliths are generally in freshwater crayfish particularly belonging to the families Astacidae and Parastacidae as a pair of hard, thick round white discs on either side of the stomach, stored between the cuticle and the epidermis of the foregut (Greenaway, 1985; Waddy, 1995) and are usually hemispherical (Figure 5) and calcium carbonate. However, it has also been detected in some marine crustaceans such as the Norwegian lobster, *Nephrops norvegicus* (Sheridan et al., 2016). Before molting, gastroliths can grow to about 8-12 mm and are similar in shape and size. Immediately after molting, the gastroliths descend into the foregut lumen and slowly dissolve there, and the contents of the dissolved gastroliths are mostly resorbed by the intestinal epithelium and hepatopancreas (Kozak et al., 2015). In terms of physical properties, gastric stones are round-convex and hemispherical with concave lower edges (Tucker, & Tucker, 2019). They look like stemless buttons or miniature button mushrooms.



Figure 5. Crayfish gastroliths (Original)

The color of gastroliths is white to slightly bluish, and a pale blue color draws attention when cut and polished. As the molting cycle progresses, gastric stones expand ((Tucke, & Tucker, 2019). Its functions mainly include the deposition of calcium compounds from the hemolymph in the form of calcium carbonate for the calcification of the exoskeleton after molting (Kozak et al., 2015; Jamil et al., 2022). The reabsorbed calcium is thus transferred to the cuticular epithelium via the hemolymph and used as a source of calcium for the hardening of the new crust (Holdich, 2002). The composition of gastroliths is mostly amorphous calcium phosphate or amorphous-looking carbonate apatite, although some are composed of more crystalline calcite (Luquet et al., 2016).

Lack of calcium availability leads to more energy expenditure to perform molting. As a result, the amount of energy mostly allocates to absorb more calcium from environment rather than taking from reserved calcium in the gastroliths or stone calcium. It leads to slower molting process leaving to crayfish vulnerable to be prey by other crayfish. This is in agreement with, the cannibalism in crayfish can be reduced by adding calcium concentration at the end. Because the crayfish has more energy reserved for shedding. Furthermore, calcium in feed can accelerate the mineralization process in shrimp (Jabbar et al., 2022).

Using a combination of microscopy techniques, XRD and FTIR analysis, the structure of the gastrolith and carbohydrate composition of crayfish can be established from a comparative perspective. In the examination of the structure of stomach stones; a) scanning electron microscope, b) X-ray analysis and c) X-

ray microanalyzer are used. Scanning electron microscopy micrographs are used in gastric stones, in gastric stone matrix protein, X-ray analysis in both crystalline (calcite) and amorphous calcium carbonates, and X-ray microanalyzer in determining the percentages of different components of calcium deposition of elements in gastric stone (Shechter et al., 2008; Habraken et al., 2015; Luquet et al., 2016; Pozebon et al., 2017).

CONCLUSION

In crayfish, gastroliths “stomach stone” represent a remarkable physiological process to conserve calcium carbonate embedded in a matrix of chitin. Just as humans need calcium for strong and healthy bones, freshwater crayfish also need calcium to protect themselves against external threats. As the crayfish grows, it periodically sheds its exoskeleton and forms a new one. They need large amounts of new calcium and chitin to start a new exoskeleton from scratch. Hormones facilitate the removal of calcium carbonate from the exoskeleton during molting in crayfish, and thus the stomach contributes to the formation of a pair of these gastrolith. After the crayfish molt, the stomach stones are resorbed and used to harden the new exoskeleton. This condition causes stomach stones only in freshwater crustaceans. Because unlike seawater, fresh water has very little dissolved calcium salts, so to retain calcium, crayfish form these small stomach stones and even eat the old exoskeleton. It has been demonstrated by previous observations that there is a possibility of hemocyanin in the gastric stone matrix formed in order to create a hard structure for calcium storage. Further investigation of this situation in the future is among the important issues.

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Chapter 11

Molecular Farming: Expanding Horizons in Plant-Based Production of Pharmaceuticals, Vaccines and Industrial Compounds

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ABSTRACT

The pharmaceutical, vaccine, and industrial compound industries have tremendous potential for molecular farming, the production of bioactive molecules from genetically modified plants. This chapter provides an introduction to molecular agriculture, including its fundamentals, techniques, applications, and implementation challenges. It discusses plant transformation, gene expression, and the optimization of production systems. Pharmaceuticals, vaccines, and industrial compounds are produced utilizing plant-based platforms. This chapter focuses on the potential for personalized pharmaceuticals and biosimilars derived from botanicals, which provide cost-effective alternatives. New developments in bioreactor systems, genome editing, synthetic biology, and non-traditional plant species are discussed. Regulatory and safety concerns are addressed, including biosafety compliance, Good Manufacturing Practices (GMP), allergenicity, and food safety evaluation. This chapter focuses on future prospects, such as personalized pharmaceuticals, plant-based biosimilars, advanced bioreactor systems, genome editing, synthetic biology integration, multi-stacked traits, and exploration of non-traditional plant species. Collaboration between researchers, industry, regulatory bodies, and the general public is essential for responsible innovation, ethics, and transparent communication. This chapter concludes with an exhaustive summary of molecular agriculture, including its fundamentals, techniques, applications, challenges, and future prospects. It is a valuable resource for researchers, industry professionals, and policymakers interested in using genetically modified plants for cost-effective and sustainable bioactive molecule production.

Keywords: Molecular farming; plant-based production; pharmaceuticals; vaccines; industrial compounds; GMO's; bioactive molecules.

INTRODUCTION

In recent years, agriculture and biotechnology have made enormous advances, pushing the boundaries of what is possible in the production of pharmaceuticals, vaccines, and industrial compounds (Ma et al. 2005). Molecular farming, a cutting-edge technique that utilizes the power of plants to produce valuable medical and industrial substances, is a ground-breaking method that has garnered significant attention and holds tremendous promise (Sohail et al. 2019). Researchers are revolutionizing the production of essential compounds by genetically modifying plants to produce complex molecules, thereby opening up new avenues for sustainable and cost-effective manufacturing (Sodhi et al. 2022).

Molecular farming, also referred to as plant molecular farming or pharming, signifies a paradigm shift in the production of valuable bioactive molecules. Historically, pharmaceuticals, vaccines, and industrial compounds have been primarily synthesized via chemical processes or extracted from limited natural resources (Khatri et al. 2017; Mohammadinejad et al. 2019; Chandran et al. 2020; Wani & Aftab 2022). However, these methods frequently have inherent disadvantages, including high costs, low yields, and environmental concerns. In contrast, molecular farming utilizes the intrinsic biological machinery of plants to function as living bioreactors, enabling the large-scale, sustainable, and scalable production of high-value compounds (Tschofen et al. 2016; Buyel 2018; Kowalczyk et al. 2022; Seem & Kaur 2023).

Utilizing vegetation in molecular agriculture has a number of advantages over conventional production techniques (Buyel 2018; Shanmugaraj et al. 2020; Ahmar et al. 2020; Schillberg et al. 2021). By capturing and converting sunlight into energy through photosynthesis, plants provide an abundant and renewable source of basic materials. In addition, plants can be grown in a variety of environments, making them adaptable to various geographical locations and reducing reliance on specific regions for production. This decentralized strategy has the potential to increase global access to essential medications and vaccines, particularly in underserved regions where conventional manufacturing facilities may be inadequate. The versatility of molecular farming is one of its greatest assets (Molina-Hidalgo et al. 2021; McNulty et al. 2021; McAlister et al. 2022). By introducing DNA coding for specific proteins or enzymes, scientists can transform plants into miniature bioreactors capable of producing a variety of pharmaceuticals, vaccines, and industrial compounds (Chiba et al. 2021; SP et al. 2021; Manan et al. 2022; Matas et al. 2023). This method has proven effective in the production of life-saving medications such as antibodies, anti-infectious vaccines, therapeutic proteins, and industrial enzymes for a variety of

applications. In addition, the capability to engineer plant-based production systems affords the opportunity to modify and optimize the properties of the produced compounds, potentially improving their efficacy, stability, and safety characteristics (Donini et al. 2019; Amiri et al. 2022; dos Santos et al. 2023). The burgeoning field of molecular agriculture is not, however, without obstacles. In order to prevent unintended consequences and protect the environment, it is of the utmost importance to assure the safety and regulatory compliance of genetically modified organisms. In addition, logistical and technical obstacles must be overcome in order to scale up production to meet global demand while preserving quality (Margolin et al. 2020; Schiermeyer 2020; Chung et al. 2022; Mičúchová et al. 2022). Nonetheless, the enormous potential of molecular farming has spurred the interest and collaboration of scientists, biotechnologists, and pharmaceutical companies around the world, thereby driving innovation and advancing the field.

In this exhaustive analysis of molecular agriculture, we investigate the revolutionary advancements, emerging technologies, and significant advances in the plant-based production of pharmaceuticals, vaccines, and industrial compounds. The objectives are to examine the principles underlying this revolutionary approach, its applications in the medical and industrial sectors, the potential benefits and challenges, and the ethical implications of this rapidly evolving field. Through this effort, we hope to shed light on the expanding horizons of molecular agriculture, illuminating a future in which plants will play a central role in the sustainable production of essential compounds for the development of human health and industry.

PRINCIPLES AND TECHNIQUES OF MOLECULAR FARMING

Molecular farming, also known as plant molecular farming or pharming, is the use of genetically modified plants as bioreactors to produce valuable bioactive molecules, such as pharmaceuticals, vaccines, and industrial compounds (Rahmat & Kang 2019; De Carlo et al. 2021; Kowalczyk et al. 2022; Kothari et al. 2023). This novel strategy utilizes the inherent biological apparatus of plants to synthesize complex proteins and compounds that are traditionally difficult or expensive to produce using other methods. Here, we delve into the principles and techniques that underpin the field of molecular farming, highlighting the key components of this transformative process.

Genetic Modification:

At the core of molecular farming is the genetic modification of plants. This involves the introduction of specific genes encoding desired proteins or

enzymes into the plant's genome (Clemente et al. 2019, Mohammadinejad et al. 2019; Fischer et al. 2020). These genes can be derived from various sources, including other plants, animals, or microorganisms. The introduced genes are meticulously chosen to ensure that the plant produces the desired molecule accurately and efficiently. Typically, genetic modification is accomplished through gene transfer, gene editing (e.g. CRISPR-Cas9), or transgenic techniques (Li et al. 2020; Gupta et al. 2021; Mushtaq et al 2021).

Promoters and Enhancers:

Promoters and enhancers play a crucial role in ensuring that the genes introduced into plants are expressed at the desirable levels and in the appropriate tissues (Ray-Jones & Spivakov 2021). Promoters are DNA sequences that regulate gene expression by determining the timing and location of gene activation. In contrast, enhancers are regulatory elements that can increase the expression of a particular gene. By meticulously selecting and engineering promoters and enhancers, scientists can optimize the yield and distribution of the target molecules by controlling their production (Jores et al. 2021; Meng et al. 2021).

Plant Transformation Methods:

Numerous transformation techniques are used to introduce foreign genes into plants. Agrobacterium-mediated transformation is a prevalent technique for transferring genes into plant cells using *Agrobacterium tumefaciens*, a naturally occurring soil bacterium (Dodds et al. 2019; Imani&Kogel 2020; Lacroix&Citovsky 2022). Biolistic transformation, also known as particle bombardment, is an additional technique in which tiny gold or tungsten particles coated with DNA are fired into plant cells with a gene cannon. These techniques permit the transfer and incorporation of foreign genes into the plant genome (Lacroix&Citovsky 2020).

Plant Expression Systems:

Once the foreign genes have been effectively integrated into the plant genome, the transformed plants serve as expression systems for the production of the desired molecules. Leaves, spores, roots, or even the entire plant can serve as platforms for expression (Xu et al. 2018; Mohammadinejad et al. 2019; Gerszberg et al. 2022). The choice of the expression system depends on factors such as the type of molecule being produced, its intended use, and the optimal conditions for production and purification.

Downstream Processing:

After the plants have produced the target molecules, downstream processing techniques are employed to extract, purify, and formulate the final product (Chen et al. 2022; Chung et al. 2022; Almeida et al. 2023). This may involve pulverizing the plant material, extracting the desired molecules using solvents or buffers, and purifying the molecules using chromatography or other separation techniques. Processing downstream guarantees that the final product satisfies the required purity, potency, and safety standards.

Regulatory Considerations:

To ensure safety and efficacy, the development and commercialization of molecular agricultural products must adhere to stringent regulatory guidelines. Regulatory agencies evaluate the potential hazards associated with genetically modified plants and the quality, safety, and stability of the produced compounds. Throughout the production process, compliance with Good Manufacturing Practices (GMP) and other pertinent regulations is essential (Elnahal et al. 2022; Hundleby et al. 2022). The principles and techniques of molecular farming have advanced the field, allowing for the production of a wide variety of pharmaceuticals, vaccines, and industrial compounds. Through genetic modification, careful selection of promoters and enhancers, plant transformation methods, selection of expression systems, downstream processing, and regulatory considerations, molecular farming has revolutionized the production landscape, providing sustainable and scalable solutions to meet the rising global demand for essential bioactive molecules (Koul 2022; Hamdan et al. 2022).

APPLICATIONS OF MOLECULAR FARMING

Molecular farming's ability to utilize plants as bioreactors for the production of valuable bioactive molecules has led to a wide range of applications in the disciplines of medicine, agriculture, and industry. This innovative approach offers numerous benefits over conventional production techniques, including cost-effectiveness, scalability, and sustainability. The following are notable applications of molecular agriculture.

Pharmaceutical Production:

The production of pharmaceuticals is one of the primary applications of molecular farming. Engineered plants can produce therapeutic proteins, antibodies, enzymes, and other bioactive compounds used to treat a variety of diseases. (Bhat et al. 2022; Bapat et al. 2023). This strategy has demonstrated tremendous potential for the production of affordable and accessible medicines,

especially in settings with limited resources. Insulin, monoclonal antibodies for cancer treatment, and vaccines against infectious diseases such as hepatitis B and influenza are examples of pharmaceuticals derived from plants (Khalid et al. 2022; Wani et al. 2022).

Vaccine Manufacturing:

Molecular farming has revolutionized vaccine production by facilitating the safe and effective production of vaccines in plants. (Gaobotse et al 2022;Stander et al. 2022). Plants can be engineered to produce viral antigens, subunit vaccines, and virus-like particles (VLPs) that elicit immune responses (Chandrasekar et al. 2022; O'Kennedy et al. 2022). This method offers several benefits, including rapid scalability, low production costs, and the capacity to produce complex vaccine formulations. Vaccines derived from plants have been created to combat viral infections such as human papillomavirus (HPV), norovirus, and even emergent diseases such as COVID-19.

Industrial Enzyme Production:

Enzymes play a crucial role in numerous industrial processes, such as the production of biofuels, textile manufacturing, and food processing. Molecular agriculture enables the production of industrial enzymes in plants, offering a sustainable and cost-effective alternative to conventional enzyme production techniques (Klocko et al. 2022; Geddes-McAlister et al. 2022). Plants can be engineered to produce enzymes with specific industrial applications, such as cellulases, amylases, and lipases (Golgeri M. Et al. 2022; Sharma et al. 2022). This strategy offers advantages such as scalability, increased stability, and decreased production costs, which contribute to greener and more efficient industrial processes.

Antibody Production:

The use of monoclonal antibodies (mAbs) has revolutionized the treatment of cancer, autoimmune disorders, and infectious diseases, among others (Shrikant et al. 2023; Verma 2023). Molecular agriculture provides a substrate for the mass production of monoclonal antibodies (mAbs) in plants, making them more accessible and affordable. The efficacy and safety profiles of plant-produced antibodies, also known as plantibodies, are comparable to those of conventionally produced antibodies. This technology has the potential to meet global demand and expand access to life-saving medications (Singh et al. 2023, Elisabeth &Toghueo 2023).

Nutraceuticals and Functional Foods:

Molecular agriculture can also be used to produce nutraceuticals and functional foods, which offer additional health benefits beyond fundamental nutrition (Kumar et al. 2022; Zhu et al. 2022). Bioactive compounds such as vitamins, antioxidants, omega-3 fatty acids, and dietary fibers can be engineered into plants. These compounds can enhance the nutritional value of food and provide potential health benefits, thereby contributing to the improvement of diets and the prevention of disease (Carpentieri et al. 2022; Sharma et al. 2023; Xu et al. 2023).

Bioremediation and phytoremediation:

The ability of plants to absorb and metabolize a variety of pollutants and contaminants makes them valuable instruments for environmental cleanup. Molecular farming techniques can be used to engineer plants with enhanced toxicity removal capabilities, a procedure known as phytoremediation (Mirzaei et al. 2022, Saravanan et al. 2022). This strategy has the potential to address soil and water contamination, reducing reliance on conventional remediation techniques. As researchers investigate new avenues and improve production processes, the applications of molecular agriculture continue to expand. This innovative approach offers sustainable and scalable solutions for the production of pharmaceuticals, vaccines, industrial compounds, and environmentally beneficial technologies by harnessing the power of plants, paving the way for a more efficient and accessible future.

**DOWNSTREAM PROCESSING AND PRODUCT PURIFICATION
IN MOLECULAR FARMING**

Downstream processing is essential to molecular agriculture because it facilitates the extraction, purification, and formulation of bioactive molecules of interest produced in plants. This phase of production guarantees that the final products satisfy the required purity, potency, and safety standards. Here are the main aspects of molecular agriculture's downstream processing and product purification.

Harvesting and Disruption:

Once the plants have produced the desired molecules, they are harvested and processed to extract the compounds of interest. The desired plant portions (leaves, fruits, seeds, etc.) can be harvested by cutting, uprooting, or mechanically separating them. Following harvesting, the plant material is disrupted in order to break down the plant cells and liberate the target

molecules. Mechanical techniques (grinding, homogenization), enzymatic therapies, and chemical treatments are all viable disruption techniques.

Clarification:

Following disruption, the extract is clarified to remove undesirable insoluble detritus, cell debris, and particles. Filtration, centrifugation, and sedimentation are all viable techniques for achieving clarification. These techniques help obtain a transparent extract that can be further refined.

Concentration and precipitation:

In some instances, the target molecules may be present in the extract at minimal concentrations. To reduce the volume and increase the concentration of the desired compounds, concentration techniques such as ultrafiltration and evaporation can be utilized. Utilizing their unique physicochemical properties (e.g., pH-dependent precipitation, salting out, or temperature-induced precipitation), precipitation techniques can also be utilized to selectively isolate and concentrate the target molecules (Yakubovich et al. 2022; Jalaludin et al. 2023; Mostashari et al. 2023).

Chromatography:

Chromatography is an important purification technique utilized in downstream processing to separate and purify target molecules from complex mixtures. Depending on the characteristics of the target molecules, various chromatographic techniques, such as affinity chromatography, ion exchange chromatography, size exclusion chromatography, and hydrophobic interaction chromatography, can be utilized (Chakraborty 2022; Matsuda 2022; González-Félix et al. 2022). Utilizing distinctions in charge, size, hydrophobicity, or specific binding interactions, these methods achieve purification and isolation.

Filtration and ultrafiltration:

The target molecule solution is filtered to remove impurities such as particles, microorganisms, and aggregates. Various pore sizes can be used for filtration, depending on the intended level of purification and the size of the impurities to be eliminated. Ultrafiltration, a more specialized filtration technique, can be used to separate molecules based on their size, removing smaller molecules or solvents while retaining the target molecules (Chung et al. 2022; Gauba et al. 2023).

Formulation and Stabilization:

Once the target molecules have been purified, they can be prepared for their intended use by undergoing formulation processes. Formulation is the process of combining purified molecules with the appropriate excipients, stabilizers, and preservatives to improve their stability, solubility, and shelf life. The formulation procedure may involve buffer exchange, sterile filtration, lyophilization (freeze-drying), or encapsulation, among other phases (Liu & Timko 2022; Mičúchová et al. 2022; Wani & Aftab 2022; Eidenberger et al. 2023).

Quality Control and Characterization:

Throughout the downstream processing and purification stages, quality control measures are implemented to ensure the final products meet the required specifications. This includes analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry, electrophoresis, spectroscopy, and bioassays to assess the purity, potency, identity, and safety of the target molecules. These tests help verify the integrity and consistency of the molecular farming products. Downstream processing and product purification in molecular farming are critical for obtaining high-quality bioactive molecules with the desired characteristics. The optimization of these processes is essential to achieving high yields, purity, and functionality while maintaining cost-effectiveness and scalability.

CHALLENGES AND LIMITATIONS OF MOLECULAR FARMING

Although molecular agriculture bears great promise for the production of valuable bioactive molecules, it is not devoid of obstacles and limitations. To ensure the success and widespread adoption of this innovative approach, these factors must be carefully considered and addressed. Here are some of the most significant obstacles and restrictions associated with molecular agriculture.

Regulatory and Safety Concerns:

Molecular agriculture's use of genetically modified vegetation raises regulatory and safety concerns. In many nations, the use of genetically modified organisms (GMOs) is strictly regulated, necessitating extensive safety and risk assessments (Rodríguez et al. 2022; Martin 2022, Mmbando 2023). In order to prevent negative effects on biodiversity and ecosystems, the potential for gene flow to untamed relatives and unintended ecological consequences must also be meticulously managed.

Contamination and Purity:

Maintaining the purity of molecular farming products and averting contamination are crucial considerations. Cross-contamination of genetically modified crops with non-GMO crops can be a cause for concern, as it may result in unintended distribution and consumer resistance. Implementing effective isolation, containment, and monitoring strategies is essential for ensuring the integrity of molecular agriculture production.

Scaling Up Production:

It can be difficult to increase molecular farming production to meet global demand. To achieve high yields of target molecules on a large scale, effective plant transformation techniques, optimal expression systems, and robust downstream processing are required. The economic viability of molecular farming depends on the development of efficient and scalable production techniques.

Protein Folding and Modification:

Protein folding and post-translational modifications may present obstacles for the synthesis of complex proteins in plants (Wang et al. 2022; Beygmoradi et al. 2023). Certain bioactive molecules, such as antibodies, require specific structuring and alterations to preserve their functionality. It is an ongoing area of research and development to ensure that plant-based expression systems can precisely fold and modify proteins to their active forms (Zheng et al. 2022; Dupuis et al. 2023).

Public Perception and Acceptance:

The public's perception and acceptance of genetically modified organisms and products can have a significant impact on the implementation of molecular agriculture. Consumption of products derived from genetically modified organisms may raise safety, ethical, and long-term health concerns. It is crucial for the acceptance and success of molecular agriculture technologies to engage in open communication and address public concerns through education and awareness.

Intellectual Property Rights:

Molecular agriculture mandates significant investments in research and development. Obtaining intellectual property rights for genetically modified plant lines, gene constructs, and production processes can be difficult and complicated. Intellectual property issues can have an impact on the accessibility

and affordability of molecular agriculture products, particularly in settings with limited resources.

Product Complexity and Cost:

Due to their complexity, certain bioactive molecules, such as complex proteins and vaccines, can make cost-effective production challenging. To assure the economic viability of molecular agriculture, it is necessary to develop efficient and optimized methods for mass production, purification, and formulation (Thanigaivel et al. 2022; Thevarajah et al. 2022). The cost-effectiveness of products derived from molecular agriculture must be meticulously evaluated against their market value and demand. Despite these obstacles and constraints, the field of molecular agriculture is progressing due to ongoing technological advances, a greater understanding of plant biology, and enhanced regulatory frameworks. These obstacles must be overcome for molecular farming to reach its full potential as a platform for the sustainable and efficient production of pharmaceuticals, vaccines, and industrial compounds.

FUTURE PROSPECTS AND EMERGING TRENDS IN MOLECULAR FARMING

Molecular farming has the potential to revolutionize many industries, including agriculture, medicine, and industry. Several future prospects and emerging trends are anticipated to influence the field's development as it advances. Here are some important growth and innovation areas in molecular agriculture.

Customized Medicines and Personalized Therapeutics:

Advances in molecular farming techniques and comprehension of plant biology have the potential to facilitate the development of personalized medicines and therapies. Plants can be engineered to produce proteins, antibodies, and vaccines that are tailored to the requirements of individual patients. This individualized approach has the potential to improve treatment outcomes, increase patient adherence, and address drug resistance issues.

Plant-Based Biosimilars:

Biosimilars, which are extremely similar versions of approved biologic drugs, can be developed utilizing plant-based production systems. Molecular farming provides a cost-effective and scalable platform for the production of biosimilars, thereby addressing the growing demand for less expensive alternatives to biologic medications. Biosimilars derived from plants have the

potential to expand access to essential treatments for cancer, autoimmune disorders, and chronic conditions, among others.

Advanced Bioreactor Systems:

Emerging trend: the creation of advanced bioreactor systems for molecular agriculture. Being investigated are bioreactors that optimize plant growth, enhance protein expression, and improve production efficiency. Bioreactor technologies, such as hydroponics, aeroponics, and vertical farming, provide precise control over growth conditions, nutrient delivery, and light exposure, resulting in increased yields and improved quality of target molecules.

Genome Editing Technologies:

Precision genome editing technologies, such as CRISPR-Cas9, have created new opportunities for molecular agriculture. These instruments allow for the targeted modification of plant genomes, enabling the precise integration of desired traits and enhanced expression of target molecules. Genome editing can improve the efficacy, speed, and accuracy of molecular agriculture, allowing for the creation of novel plant-based production systems.

Integration of Synthetic Biology:

The incorporation of synthetic biology techniques into molecular agriculture is an emerging trend with considerable potential. Synthetic biology techniques enable the design and construction of novel genetic circuits and metabolic pathways in plants, thereby improving their capacity to produce complex bioactive molecules. Synthetic biology can improve plant physiology, fine-tune gene expression, and allow for the production of molecules with enhanced functionality or novel properties.

Multi-Stacked Traits and Crop Improvement:

Molecular agriculture can be combined with conventional crop enhancement techniques to create plants with multiple traits. Researchers can enhance plant productivity, disease resistance, and nutritional value by introducing multiple genomes. This comprehensive strategy has the potential to increase crop yields, optimize resource utilization, and address global issues such as food security and sustainable agriculture.

Expansion into Non-Traditional Plant Species:

While the majority of efforts in molecular agriculture have been concentrated on well-established plant species, there is a growing interest in

investigating non-traditional plant species for molecular agriculture. This includes plants with unique characteristics, rapid growth rates, or high environmental tolerance. Increasing the variety of plant species utilized in molecular agriculture could create new opportunities for production scalability, resource efficiency, and product diversity.

Environmental Remediation and Bio-Based Industries:

Molecular agriculture can be utilized for environmental remediation and bio-based compound production. Through phytoremediation or the production of industrial compounds, biofuels, and biomaterials, plants can be engineered to efficiently remove pollutants from the environment. This convergence of molecular agriculture and bio-based industries offers sustainable solutions to environmental problems and facilitates the transition to a greener economy. As molecular farming research and development advances, these future prospects and emerging trends are anticipated to influence the trajectory of the field. Technology advancements, the integration of synthetic biology, and the optimization of plant-based production systems will contribute to the full potential of molecular farming in the production of pharmaceuticals, vaccines, industrial compounds, and beyond.

CONCLUSION

In conclusion, molecular agriculture is a revolutionary method for producing valuable bioactive molecules from genetically modified plants. It contains great potential for expanding the horizons of plant-based pharmaceutical, vaccine, and industrial compound production. The principles and techniques of molecular agriculture, such as plant transformation, gene expression, and production system optimization, provide a potent arsenal for maximizing the biofactory potential of plants. From the production of therapeutic proteins and antibodies to the development of biofuels and bioplastics, the applications of molecular agriculture are extensive and varied. Utilizing the distinct advantages of plants, such as their ability to synthesize complex molecules and their scalability, molecular farming provides a sustainable and cost-effective alternative to conventional production techniques. However, molecular agriculture is not devoid of obstacles and limitations. To ensure the responsible development and commercialization of genetically modified organisms, regulatory factors and safety concerns are of the utmost importance. The necessity of adhering to biosafety regulations, rigorous risk assessments, and Good Manufacturing Practices (GMP) highlights the commitment to safety, environmental sustainability, and consumer confidence. Despite these obstacles,

ongoing innovations and emerging trends in molecular agriculture are likely to influence its future prospects. Customized pharmaceuticals, plant-based biosimilars, advanced bioreactor systems, genome editing technologies, synthetic biology integration, multi-stacked traits, and the investigation of non-traditional plant species all contribute to the expansion of molecular farming's horizons. By embracing these future prospects and emerging trends, molecular agriculture has the potential to revolutionize industries, enhance healthcare outcomes, address environmental challenges, and contribute to a sustainable and bio-based economy. As the field of molecular agriculture continues to develop, it is crucial to foster collaboration between researchers, industry, regulatory bodies, and the public in order to ensure responsible innovation, ethical considerations, and open communication. By doing so, we can realize the transformative effects of molecular farming on global health, agriculture, and industry, and unleash its full potential.

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Chapter 12

Contemporary Approaches to Food Safety: Risks, Innovations and Future Perspectives

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ABSTRACT

Food safety is a discipline that assesses and manages risks in the production, processing, distribution and consumption of food to ensure that people have access to safe, healthy and edible food. This discipline aims to ensure that food is protected against hazards arising from biological, chemical and physical risk factors.

Foodborne disease prevention aims to prevent microbial contamination through hygiene and sanitation practices. In addition, innovative food processing techniques involve the use of advanced technologies to inactivate microorganisms in foods. Methods such as high- pressure processing (HPP) and pasteurization are effective in reducing the impact of microorganisms, while ultraviolet (UV) irradiation and ozonation also help to control microorganisms. In addition, techniques such as electron beam (E-beam) and radiation are among the innovative methods used in food processing. Technological innovations provide improvements in food safety processes through advanced technologies such as rapid detection methods, biosensors and artificial intelligence. In this way, potential risk factors in food can be detected more effectively and measures can be taken. In addition, the use of technologies such as the Internet of Things (IoT) can improve traceability and efficiency in food safety processes.

The future of food safety includes innovative solutions such as genetic engineering, anti-counterfeiting, laboratory-produced foods and sustainable food sources. These approaches contribute to the development of more effective and sustainable solutions in food safety. This study aims to develop a safer, healthier and sustainable food system by emphasizing food safety and risk assessment, prevention of foodborne diseases, innovative food processing techniques, technological innovations and the future of food safety. In this framework, key steps need to be taken, such as risk analysis, implementation of hygiene standards, use of technological innovations and adoption of innovative solutions.

Keywords: Food Safety, Risk Assessment, Innovative Food Processing Techniques, Technological Innovations, Future Perspectives

INTRODUCTION

Food safety is a fundamental requirement for a healthy life and is of great importance to protect the health of consumers. The concept of food safety includes the protection of food against any biological, chemical or physical risk factors during the production, processing, distribution and consumption of food. In this chapter, we will provide an in-depth understanding of food safety and risk assessment and discuss current innovations.

First, we will focus on the importance of food safety. Consumer access to healthy food is a critical factor in nutrition and health. The prevalence of foodborne diseases emphasizes the need for food safety measures. Moreover, the increase in international trade and the complexity of the food supply chain call for even greater vigilance on food safety. Risk analysis, risk assessment and risk communication are fundamental principles for effective food safety management.

In this study, we will also discuss the impact of innovative food processing techniques on food safety, including technologies such as high pressure processing (HPP), pasteurization techniques, ultraviolet (UV) irradiation, ozonation, electron beam (E-beam) and radiation. Technological innovations in food safety will also be covered. Rapid detection methods and molecular analysis help to quickly and accurately detect potential hazards in food. Biosensors and nanotechnology-based solutions offer more sensitive and precise analysis methods. Furthermore, technologies such as the Internet of Things (IoT) and artificial intelligence (AI) can play an important role in food safety management.

Finally, we will focus on the future of food safety. Genetic engineering and food safety offer a new perspective and potential opportunities to make food safer and more nutritious. Furthermore, when combating counterfeiting and food fraud, it is important to use new technologies to ensure reliability and traceability. Laboratory-grown foods and new food sources may also play an important role in food safety in the future. This book chapter covers the most fundamental concepts, current innovations and future trends in food safety and risk assessment. Adopting an informed and knowledgeable approach to food safety will contribute to increasing the consumption of healthy and safe food and protecting the well-being of societies.

Food Safety and Risk Assessment

Food safety is a very important issue that aims to prevent potential health risks by preventing foodborne diseases and ensuring the safe consumption of food. It covers the control of biological, chemical and physical hazards that may arise during the production, processing, transportation, storage and consumption of food. The concept of food safety involves identifying the potential risks of

foodborne diseases and taking preventive measures to reduce these risks. Foodborne diseases are health problems caused by microorganisms, toxins or chemicals that can affect humans. These diseases can cause symptoms such as diarrhea, nausea, vomiting and fever, and in severe cases can be life-threatening. Food safety measures are therefore of great public health importance. The importance of food safety is emphasized by numerous international sources (FAO, 2019).

International organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have developed regulations and standards at national and international level to ensure and protect food safety. These standards provide guidelines for identifying food safety risks, managing risks and protecting consumers. Food safety management encompasses a set of procedures that enable food businesses and producers to fulfill their responsibilities to ensure food safety. A commonly used method is the Hazard Analysis and Critical Control Points (HACCP) system. HACCP helps food businesses to analyze their production processes, identify hazards and determine critical control points. Risk assessment plays a vital role in food safety by identifying and assessing the potential risks associated with foodborne hazards. This assessment involves a scientific approach based on microbiological analysis, chemical analysis and other scientific methods. Consumer awareness and attitudes towards food safety are also crucial. Informed consumer behavior increases awareness of proper food storage, hygienic preparation methods and safe supply chain practices. Therefore, educating and raising awareness of consumers plays an important role in ensuring food safety. Internationally developed regulations, standards and management systems contribute to improvements in food safety. However, considering the ever-evolving technology and innovations in the field, continuous efforts are required to reduce risks and increase consumer confidence in food safety (FAO, 2018; WHO, 2019).

Risk analysis plays a crucial role in ensuring food safety by identifying, assessing and communicating potential hazards associated with food production, processing and consumption. This process involves three key components: risk analysis, risk assessment and risk communication. Understanding these concepts and the links between them is essential for the effective management of food safety risks. Risk analysis is a systematic approach to identifying, assessing and managing food safety risks. It involves a series of interlinked steps, including risk assessment, risk management and risk communication. The purpose of risk analysis is to provide a scientific basis for decision-making and to promote the protection of public health. Risk assessment is a component of risk analysis that focuses on the scientific evaluation of potential hazards and the estimation of their

probability and consequences. It involves four basic steps: hazard identification, hazard characterization, exposure assessment and risk characterization. Through risk assessment, the potential risks associated with specific hazards in the food chain are identified and quantified. Risk communication is a vital aspect of risk analysis, involving the exchange of information about risks and their management among stakeholders, including consumers, producers, regulators and scientists. Effective risk communication ensures that accurate, timely and relevant information is communicated to facilitate informed decision-making and public understanding of food safety risks. It includes transparent and interactive communication methods that address public concerns, build trust and support risk mitigation strategies. Risk analysis, risk assessment and risk communication are interlinked components that underpin effective food safety management. These processes facilitate the identification, assessment and communication of potential risks associated with food production and consumption. Using a systematic and science-based approach, stakeholders can make informed decisions, implement appropriate risk management strategies and ensure the protection of public health (Anonymous, 1; Anonymous 2, Anonymous 3).

Biological, chemical and physical risk factors may occur during the production, processing, distribution and consumption of food. These risk factors can affect food quality and pose a threat to consumer health (Çetin and Şahin, 2017; Çopuroğlu et al., 2015).

Biological risk factors refer to the potential hazards associated with food-associated microorganisms and microbial pathogens. They can cause foodborne infections and poisoning. These microorganisms can be found at various stages of food production, processing plants, farms and points of consumption. Examples of biological risk factors include bacteria such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, viruses such as Norovirus and Hepatitis A, and parasites such as *Toxoplasma gondii* (Erkmen, 2010).

Chemical risk factors arise due to natural toxins present in foods or contaminants from various sources. Factors such as pesticides, industrial pollutants, environmental pollutants, packaging materials and cleaning agents can contaminate food and pose health risks. Examples of chemicals that may have adverse effects and potential health problems include aflatoxins, pesticide residues, heavy metals and nitrates (Güler and Can, 2017).

Physical risk factors are caused by foreign bodies or various physical agents present in food. These substances can accidentally mix with food during production, processing, packaging and consumption. Foreign bodies in food can cause choking, injuries or broken teeth. Examples of foreign bodies that may be present in food as physical risk factors include glass fragments, metal fragments,

plastic particles and sawdust. The identification, assessment and management of these risk factors is very important and is emphasized by the food industry, governments and international organizations. Designing food safety policies and regulations that address these risk factors is of great importance in protecting consumer health (Çetin and Şahin, 2017).

Prevention of Foodborne Diseases

Foodborne diseases are a major public health concern worldwide. These diseases are caused by pathogens, toxins and other harmful substances found in foods available for human consumption (Anonymous, 4). Prevention of foodborne diseases is of great importance to protect the health of both individuals and communities. Prevention of foodborne diseases requires a variety of measures and strategies. These include factors such as the implementation of hygienic food production, processing and storage methods, safe food supply, labeling and following regulatory standards. In addition, awareness-raising efforts, education programs and effective communication strategies by public health agencies and health professionals also play a critical role in disease prevention. Implementing strategies such as hygienic production processes, effective inspections, education and awareness-raising efforts will help us achieve this important goal by promoting safe and healthy food consumption (Redmond and Griffith, 2003; Tod et al., 2008; WHO, 2019).

Prevention of foodborne diseases requires a multidisciplinary approach. Cooperation and coordination should be ensured between food producers, businesses, inspection units, health institutions and consumers. Agriculture, food safety policies, public health policies and regulatory measures also play an important role in this process. In addition, scientific research is also of great importance for the prevention of foodborne diseases. Studies in areas such as microbiology, toxicology and epidemiology help us understand the causes of foodborne diseases and develop effective measures. These studies provide essential information for the formulation and implementation of food safety policies (Medeiros et al., 2001; Jay et al., 2008; Doyle et al., 2020).

Microbial pathogens are microorganisms that can cause disease when ingested through contaminated food (Mead et al., 1999). These pathogens can be found in a variety of food sources and can cause a wide range of illnesses, from mild gastrointestinal discomfort to serious and life-threatening conditions. This article aims to provide a comprehensive overview of microbial pathogens commonly associated with foodborne infections, their sources, transmission routes and preventive measures (CDC, 2020). Bacterial pathogens are one of the leading causes of foodborne infections. They can contaminate food in a variety of ways,

including agricultural practices, processing, transportation and improper storage. Examples of bacterial pathogens include *Salmonella*, *Campylobacter*, *Escherichia coli* (*E. coli*), *Listeria monocytogenes* and *Staphylococcus aureus*. These pathogens can cause diseases such as salmonellosis, campylobacteriosis, *E. coli* infections, listeriosis and staphylococcal food poisoning.

Viral pathogens are another important group of microorganisms responsible for foodborne infections. These pathogens can be introduced into food through infected individuals, contaminated water sources or improper handling practices. Common viral pathogens associated with foodborne illness include Norovirus, Hepatitis A virus and Rotavirus. These viruses can cause gastrointestinal symptoms such as vomiting, diarrhea and abdominal pain.

Parasites can also be a source of foodborne infections. These pathogens are often associated with consumption of contaminated water or undercooked/raw food. Examples of parasitic pathogens include *Cryptosporidium*, *Giardia*, *Toxoplasma gondii* and *Trichinella*. These parasites can cause diseases such as cryptosporidiosis, giardiasis, toxoplasmosis and trichinellosis.

Preventing foodborne infections requires a multifaceted approach that includes a variety of preventive measures. These measures include:

- *Good agricultural practices to minimize pollution at source.

- *Appropriate food processing, storage and cooking practices to prevent cross-contamination and kill pathogens.

- *Application of Hazard Analysis and Critical Control Points (HACCP) systems in food production and processing.

- *Adequate sanitation and hygiene practices in food businesses and homes.

- *Education and awareness programs to promote safe food handling and consumption practices (CDC, 2020).

Microbial pathogens play an important role in foodborne infections and cause a significant burden on public health. Understanding the sources, transmission routes and preventive measures associated with these pathogens is essential for the development of effective strategies to reduce the risk of foodborne illness. It is essential that food producers, regulators and consumers work together to ensure the safety of the food supply chain and protect public health (WHO, 2015).

Food poisoning is a common public health problem caused by the consumption of contaminated or poisoned food. It is a disease caused by ingestion of harmful microorganisms or toxins found in food (CDC, 2020). Microbial food poisoning occurs when harmful bacteria, viruses, parasites or fungi contaminate food and then cause illness when ingested. Common microbial pathogens involved in food poisoning include *Salmonella*, *Campylobacter*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, *Clostridium botulinum*, and Norovirus" (CDC, 2020).

Toxin-mediated food poisoning refers to the ingestion of preformed toxins found in contaminated food. Certain bacteria, such as *Staphylococcus aureus* and *Bacillus cereus*, produce toxins that can withstand cooking temperatures and cause illness when consumed.

Food poisoning can manifest in various ways depending on the causative agent and the toxin involved. Common symptoms include nausea, vomiting, diarrhea, abdominal pain, fever and in severe cases dehydration and neurological symptoms. Prevention is key to reducing the incidence of food poisoning. Effective measures include: proper food handling, storage and preparation to prevent cross-contamination and bacterial growth; ensuring good personal hygiene, including hand washing and proper sanitation practices; ensuring food safety throughout the entire supply chain, including production, processing, transportation and storage; implementing Hazard Analysis and Critical Control Points (HACCP) systems to identify and control potential hazards; conducting regular inspections and audits of food establishments to ensure compliance with food safety regulations (CDC, 2020; WHO, 2015; Todd et al, 2008).

Hygiene and sanitation practices are essential to prevent contamination of food by harmful microorganisms, chemicals and physical hazards. They help minimize the risk of foodborne illness and maintain the quality and integrity of food products. Effective hygiene and sanitation practices are vital at all stages of the food production chain, from farm to fork (WHO, 2006; FDA, 2017; FSA, 2021).

Personal hygiene is essential to prevent the introduction and spread of pathogens. Key aspects of personal hygiene include:

Hand washing Wash hands thoroughly and frequently with soap and water before touching food, after using the toilet, after touching raw food and after handling waste or chemicals.

Personal Protective Equipment (PPE): The use of appropriate PPE such as gloves, aprons and hairnets helps prevent contamination from human contact.

Disease and Injury Management: Food handlers should report illnesses or injuries that could potentially compromise food safety and avoid handling food when symptoms such as diarrhea, vomiting or respiratory infections are present.

Adopting appropriate practices in **food handling and storage** processes, preventing contamination and ensuring food safety is of utmost importance. These important considerations include:

Prevent Cross Contamination: Separate raw and cooked foods, use separate containers and cutting boards, and avoid contact between raw juices and other foodstuffs.

Temperature Control: Adhering to appropriate temperature control measures,

including refrigeration and cooking temperatures, to prevent bacterial growth and minimize the risk of foodborne illness.

Proper Storage: Ensuring proper storage conditions to maintain food quality and safety, such as maintaining the right temperatures and preventing exposure to pests.

Cleaning and Sanitation: vital for preventing the growth and spread of pathogens. Important considerations include:

Cleaning Procedures: Establish regular cleaning schedules, use appropriate cleaning agents and properly clean food contact surfaces, equipment and containers.

Sanitization Practices: Application of sanitizing agents such as chlorine or quaternary ammonium compounds to eliminate or reduce microbial contamination on surfaces.

Equipment Maintenance: Regular inspection, cleaning and maintenance of equipment to prevent accumulation of dirt, debris and microbial growth.

Education and training: Appropriate education and training of food handlers and workers is fundamental to promoting and ensuring adherence to hygiene and sanitation practices. Training programs should cover topics such as personal hygiene, safe food handling, cleaning procedures and the importance of preventing cross-contamination (WHO, 2006; FDA, 2017; FSA, 2021; EFSA, 2017, Luber, 2009).

Hygiene and sanitation practices are pillars of food safety. By implementing and maintaining strong hygiene and sanitation protocols, the risk of foodborne illness can be significantly reduced. Adherence to proper practices, training and continuous monitoring are key to maintaining high hygiene standards and protecting public health (FSA, 2021).

Technological Innovations in Food Safety

Technological innovations in food safety include various methods used to reduce risks and ensure the safety of food products during food processing, storage, packaging and transportation.

Food processing techniques are an important area in the food industry that involves the preparation, preservation and processing of products (Barbosa-Cánovas et al., 2004). Innovative food processing techniques aim to improve the quality, safety and nutritional value of foods by going beyond traditional methods (Rastogi and Knorr, 2013). These techniques can improve the efficiency of food processing, optimize production processes and enable the development of new products (Gavahian et al., 2020). Innovative food processing techniques include high-pressure processing (HPP), pulsed electric fields (PEF), cold plasma (CP),

supercritical carbon dioxide (SC- CO₂), and ultrasound (USN) processing. These techniques can be used for different purposes such as reducing microbial activity in food products, controlling enzymatic activity, improving nutritional content, and extending the shelf life of products (Gavahian et al., 2020; Raso and Barbosa-Cánovas, 2003; Rastogi and Knorr, 2013).

Some technological innovations used in food safety:

High Pressure Processing (HPP): A technology that inactivates microorganisms by applying high pressure to food products. HPP can inactivate pathogens while maintaining nutritional value and quality.

High pressure processing (HPP) and pasteurization are two important processing techniques widely used in the food industry to control microorganisms and make products safe (Balasubramaniam et al., 2016; Erkmen and Bozoglu, 2016). HPP is a technique that allows food products to be processed under high hydrostatic pressure. This process is used to inactivate microorganisms, control enzymatic activity and extend the shelf life of food products (Balasubramaniam et al., 2016). Unlike conventional heat treatments, HPP occurs at low temperature, which allows for the preservation of nutritional value and better preservation of the natural taste, color and texture of products (Oey et al., 2008). Furthermore, HPP is considered as an alternative that can be used when sterilization of foods is not required (Balny et al., 2002). On the other hand, pasteurization is a process that involves heating foods to control microorganisms. This process reduces the microbial load of food products while ensuring safe consumption without affecting the quality and nutritional value of the product (Erkmen and Bozoglu, 2016). Pasteurization is usually carried out at low temperatures and for short periods of time. In this way, the growth of microorganisms is controlled and pathogens are killed, while the nutritional value of foods is preserved (Park et al., 2014). Both methods are widely used in a variety of food products, especially sensitive products such as fresh fruits and vegetables, meat products, juices and milk.

Microbial Inactivation Technologies: Technologies such as ultraviolet (UV) rays, electron beam, radiation provide microbial inactivation by disrupting the DNA and cellular components of microorganisms. These methods are used to reduce microbial contamination and extend the shelf life of food products. Ultraviolet (UV) irradiation and ozonation are effective disinfection methods widely used in the food industry (Andreozzi et al., 1999; Calle et al., 2021; Yucel Sengun and Kendirci, 2018). These technologies are critical for preventing microbial contamination and ensuring food safety. Both UV irradiation and ozonation are used to extend the shelf life of food products and provide safe products to consumers by inactivating microorganisms (Calle et al., 2021; Kim

et al., 2023). UV irradiation uses ultraviolet rays to inactivate microorganisms by affecting their DNA, RNA and proteins (Kim et al., 2023). UV rays help to control pathogens and other harmful microorganisms by inhibiting the reproduction and growth of microorganisms. This is an effective method for reducing microbial contamination on the surfaces of food products, packaging materials and water systems. UV irradiation offers an environmentally friendly and residue-free option without the use of chemical disinfectants (Calle et al., 2021; Kim et al., 2009). On the other hand, ozonation is a process that uses ozone gas to inactivate microorganisms (Yucel Sengun and Kendirci, 2018). Ozone is a powerful oxidizing agent and acts by breaking down the cell membrane of microorganisms or disrupting their enzymatic systems. Ozonation is used both in water-based systems and on the surfaces of food products to reduce the microbial load and provide disinfection without residue. Furthermore, ozonation can extend the shelf life of food products by preventing their oxidation and spoilage (Andreozzi et al., 1999; Gutierrez and Rodriguez, 2019). UV irradiation and ozonation are important tools for maintaining hygiene and controlling microbial contamination in the food industry. These technologies contribute to environmental sustainability by reducing the use of chemical disinfectants while improving food safety. However, the effectiveness of these processes depends on many factors, such as the application conditions and the characteristics of the food product. Therefore, it is important to determine the correct parameters and take appropriate control measures (Kim et al., 2023; Yucel Sengun and Kendirci, 2018; Wang and Wu, 2023).

Electron beam (E-beam) and radiation applications:

Electron beam (E-beam) and radiation applications are technologies widely used in the food industry for disinfection and quality improvement of processed products (Raso and Barbosa-Cánovas, 2003; Aaliya et al., 2021). These methods provide significant advantages such as reducing microbial contamination, controlling insect pests and extending the shelf life of food products (Raso and Barbosa-Cánovas, 2003; Aaliya et al., 2021). Electron irradiation is a process that uses high-energy electrons and is applied to the surfaces or packages of food products. The electrons cause microbial inactivation by damaging the cell membranes of microorganisms or disrupting their DNA. This method is notable as a disinfection method that does not require thermal treatment and has minimal impact on the nutritional value and sensory properties of food products. Furthermore, electron beam treatment can be effective in the control of insect pests (Raso and Barbosa- Cánovas, 2003).

Radiation applications include the treatment of food products with non-

ionizing and ionizing radiation sources (Nawrot et al., 2019). Irradiation is used to reduce the microbial load of food products and control insect pests. Radiation sources, such as gamma rays or X-rays, disrupt the DNA and other cellular components of microorganisms, resulting in microbial inactivation. Radiation treatment is particularly preferred for sensitive food products as a disinfection method that does not require thermal treatment (Nawrot et al., 2019).

Plasma Technologies: The conversion of gases into high-energy plasmas under atmospheric pressure can be effective in reducing microbial contamination. Plasma provides disinfection by damaging the cell walls of microorganisms or disrupting their DNA (Nwabor et al., 2022).

The advantages of innovative food processing techniques include short processing times, energy and water savings, nutrient preservation, and improved aroma and flavor profiles. They also offer the food industry great potential to develop a variety of new products and provide consumers with innovative experiences. Innovative food processing techniques also present some challenges compared to traditional methods. For the implementation of these techniques on a commercial scale, factors such as high investment costs, complexity of equipment and optimization of operating processes need to be taken into account. In addition, the impact of these techniques on food quality and safety should also be thoroughly assessed. Innovative food processing techniques have the potential to provide sustainability and competitive advantage in the food industry. Researchers and industry professionals are continuously working to better understand and apply the potential of these techniques (Barbosa-Cánovas et al., 2005; Toepfl et al., 2007; Delfiya et al., 2022; Gavahian et al., 2020).

Advanced Detection Methods: Technologies such as optical sensors, electrochemical sensors, magnetic resonance imaging (MRI) and spectroscopy are used to detect potential contaminants, pathogens or residues in food products. These sensors enable fast, sensitive and reliable results. Rapid detection methods are techniques used to rapidly detect potentially risky substances (pathogens, toxins, etc.). These methods offer significant advantages in terms of food safety because they are characterized by rapid results, low cost and portability. Immunologically based methods are techniques that use antibodies and are based on antigen-antibody reactions. These methods are widely used for the rapid detection of pathogens or toxins in foods. For example, immunochromatographic assays are used to rapidly detect specific analytes (e.g. pathogens such as Salmonella or E. coli) using portable devices (Abdalkhai et al., 2014; Xue et al., 2021; Chen et al., 2016; Rubab et al., 2018; Lv et al., 2020; Dhiman and Mukherjee 2021; Hussain et al., 2022).

DNA and RNA Based Diagnostics: DNA and RNA-based diagnostics are

molecular biology techniques used for the rapid and sensitive detection of pathogens or food allergens in food products. These methods are widely used in food safety analysis and have become an important tool in the food industry. Polymerase Chain Reaction (PCR) is a method of selectively amplifying target regions of DNA and RNA molecules. PCR is used to detect the presence of specific pathogens or genetic material using specific primers. This method is characterized by rapid results, high sensitivity and selectivity. DNA microarrays are used to quickly and simultaneously analyze a large number of targets for genetic material in food products.

Microarrays contain thousands of DNA probes attached to a glass or silicon surface, and this set of probes is used to determine the presence of different pathogens or genetic traits. This method provides the ability to detect multiple analytes simultaneously and offers high throughput. Next Generation Sequencing (NGS) is a technology used to completely sequence the genetic material in food products. NGS enables millions of short DNA or RNA sequences to be sequenced simultaneously. This makes it possible to detect and analyze complex mixtures of pathogens or genetic variations. NGS offers high resolution in food safety analysis, providing comprehensive data and building a large genetic knowledge base. These molecular biology techniques make it possible to perform rapid and precise analysis for food safety. This gives the food industry the advantage of improving contamination detection, rapid decision-making and quality assurance (Lee et al., 2019; Quintela et al., 2022; Im et al., 2021; Jagadeesan et al., 2019).

Smart Labeling and Tracking Systems: Smart labeling and tracking systems play an important role in food safety. With the use of technologies such as RFID (Radio Frequency Identification), NFC (Near Field Communication) and barcodes, tracking and tracing of food products is becoming easier. These systems help identify where products are, under what conditions they are transported and when they change hands at each stage in the supply chain. This makes it possible to quickly identify and isolate affected products in the event of foodborne outbreaks or recalls. It also provides consumers with reliable information and helps raise awareness about the correct handling and storage of products. Through the use of these technologies, the food industry and regulatory agencies can improve food safety by providing more effective risk management (Yu et al., 2022; Liu et al., 2019; Singh et al., 2021; Ben-Daya et al., 2020; Dasaklis et al., 2022).

Data Analytics and Monitoring: Technologies such as big data analytics, artificial intelligence and machine learning are used to collect, analyze and monitor data in food production processes and the supply chain. This makes it

possible to identify potential risks in advance and take preventive measures. First, through big data analytics, large data sets generated throughout the food production and supply chain are analyzed. As a result of these analyses, situations such as potential risks in production processes, production errors, contamination or quality problems can be identified. This information enables rapid decision-making and preventive measures. Artificial intelligence and machine learning are used to analyze data in food production and the supply chain to identify patterns, trends and anomalies. For example, machine learning algorithms can detect early signs of a production defect or quality issue by analyzing sensor data. Furthermore, AI-based systems are also used in areas such as image processing, object recognition and quality control for food safety control (Chen et al., 2021; Jin et al., 2020; Zhou et al., 2019; Zhao et al., 2019; Bongomin et al., 2019). With the use of data analytics and monitoring technologies, the food industry gains the following advantages:

- Identifying potential risks in advance and taking preventive measures.
- Improving quality control and quality assurance processes.
- Reduced production errors and increased productivity.
- Rapid detection of foodborne outbreaks and recalls.
- Ensuring product traceability and increasing consumer confidence.

Nanotechnology Applications: The use of nanomaterials on food products is emerging as a new approach to improve food safety. Nano-sized antimicrobial particles can prevent the growth of microorganisms in food products and reduce the risk of contamination. These particles are usually composed of substances with antimicrobial properties such as silver, zinc oxide, titanium dioxide. Thanks to their nano-size, these particles can be easily applied to surfaces and effectively inhibit the growth of microorganisms. For example, nano-sized antimicrobial particles in food packaging can extend the shelf life of food and reduce the risk of microbial contamination. The use of nanomaterials can also improve the quality and safety of food products. Nano-sized sensors can be used to monitor the freshness and contamination status of foods. These sensors can measure parameters such as pH, temperature, oxidation level in foods and help to detect potential risks in advance. Nanotechnology also plays an important role in food packaging. Nanocomposite materials can create packaging with more durable and barrier properties that ensure the protection of food products. In this way, the exposure of food products to external factors (light, oxygen, moisture, etc.) is reduced and the quality of the products is maintained (Ashfaq et al., 2022; Shafiq et al., 2020).

Advanced Packaging Technologies: Active and smart packaging are technological innovations used to extend the shelf life of food products, maintain quality and inform the consumer. Features such as gas control, moisture control, antimicrobial properties are used in packaging to improve food safety. Gas control is a method of controlling the composition and concentration of gases inside the packaging. For example, oxygen absorbents or ethylene absorbents can absorb gases such as oxygen or ethylene in packaging, delaying the oxidation and ripening of food products. This can extend the shelf life of food products and preserve their freshness. Moisture control is a method of regulating the moisture level inside the packaging. By using moisture absorbents, moisture absorbing materials, the amount of moisture in the packaging can be controlled. In this way, the humidity of food products can be reduced, reducing the risk of spoilage. Packaging with antimicrobial properties is used to prevent the growth and spread of microorganisms. Packaging with special coatings or nano-sized antimicrobial particles can reduce microorganism contamination on the surface of food products and improve their safety. Smart packaging makes it possible to monitor food products and provide information to consumers. For example, time and temperature indicators can help to check the suitability of food products by showing the temperature inside the package or the conditions exposed over time. Thus, consumers can be informed to choose fresh and safe food products (Omerović et al., 2021; Singh et al., 2021; Kumari et al., 2022).

Traceability and Blockchain Technology: Blockchain technology is used to trace food sources and the supply chain. This technology enables tracking the source, production process, transportation and storage conditions of food products and provides reliable information to the consumer. Blockchain technology is a decentralized digital ledger system. It enables transparent recording and sharing of transactions and data between food producers, suppliers, logistics companies and retailers. Each transaction is attached to packets of data, called blocks, and sealed with a timestamp. These blocks form an interconnected chain, making it virtually impossible to change or delete information. Blockchain technology is used to track the origin, production process, transportation and storage conditions of food products. Manufacturers can assign a unique digital ID to each food product and record all relevant information on this ID. This includes important data such as production date, production location, growing methods, fertilizers or pesticides used, transportation and storage temperatures. By scanning the digital identity of the food product on the blockchain, consumers can access detailed information about the product. Thus, they have verifiable information about factors such as the safety, quality, organic or sustainability of the food. Blockchain technology helps reduce counterfeiting, food fraud and

supply chain issues (Singh et al., 2023; Lim et al., 2021; Rejeb et al., 2020).

Internet of Things (IoT) and artificial intelligence (AI) applications: Food production processes and the supply chain are becoming traceable through sensors and devices connected to the Internet of Things (IoT). Sensors collect data in production facilities, fields and logistics networks and transmit it to the communication network in real time. This data is used to monitor and evaluate important parameters such as production conditions, storage temperatures, moisture levels, transportation time, etc. IoT provides a huge advantage in identifying potential risks to food safety in advance and taking preventive measures. For example, a food production facility can monitor adverse changes in the production environment through temperature and humidity sensors and quickly detect and respond to these situations. Thus, potential risks such as microbial growth or food spoilage can be prevented in advance. Artificial intelligence (AI) applications also play an important role in food safety. AI can analyze big data, build predictive models and perform risk assessments. By feeding food production and supply chain data into AI algorithms, it becomes possible to detect anomalies, identify patterns and predict potential risks. AI is also used in methods for food quality control and pathogen detection. For example, image processing and machine learning techniques can detect defects or detect the presence of pathogens by analyzing images of food products. However, there are also some challenges associated with the use of IoT and AI in food safety. Issues such as data security, privacy and standardization need to be considered. Furthermore, it is important to reduce costs and improve staff skills for technology deployment (Bouzembrak et al., 2019; Monteiro et al., 2021; Ben-Daya et al., 2020).

The Future of Food Safety

One of the factors expected to be at the forefront of food safety in the future is the use of innovative technologies and automation. Research shows that advances such as sensor technologies, artificial intelligence, big data analytics and automated control systems will enable more effective monitoring and control of food production, processing and distribution processes (Food and Agriculture Organization, 2018).

For example, production lines equipped with smart sensors will allow food products to be continuously monitored for quality, integrity and hygiene, while artificial intelligence algorithms will help analyze data and detect faulty production or safety risks (Hasanah and Indrawan, 2020). In this way, food producers and businesses will be able to identify potential problems at an early stage and take precautions. The use of traceability and tracking systems in the

food supply chain is also of great importance for food safety. Tools such as Internet of Things (IoT) technology, smart labels, QR codes and blockchain will be used to track the journey of food products from source to consumer and quickly identify potential safety risks (Xiong et al., 2020). In this way, consumers will have access to more information about the origin, production methods and storage conditions of food products and will be able to make more informed food choices.

Another approach that is expected to be used more in food security in the future is risk-based approaches. This approach ensures that resources are properly directed based on the assessment and prioritization of risks. Technologies such as artificial intelligence and big data analytics can help build risk-based models for food safety (Kakani et al., 2020). These models identify potential risks, enabling efficient use of resources and making interventions more targeted and effective. In this way, innovative technologies and factors such as automation, traceability and tracking systems, risk-based approaches are important elements that will shape the future of food safety. The implementation of these approaches aims to reduce foodborne diseases, prevent food fraud and increase consumer access to safe food. However, in the process of developing and implementing these technologies, it is important to consider issues such as costs, standards, data security and ethical issues.

Genetic engineering plays an important role in food safety as a branch of modern biotechnology. This technology enables the development of desired traits in plants and animals through modifications to genetic material. Genetic engineering provides the opportunity to modify genetic material to improve desired traits in plants. For example, pesticide-resistant plants, insect and disease-resistant plants, plants with high nutritional value can be obtained through genetic engineering. However, genetically engineered plants need to be carefully assessed for food safety. Therefore, risk analysis and assessment is an important step in the evaluation of genetically engineered plants in terms of food safety. Genetic engineering is also used in animals to improve desired traits. For example, traits such as disease-resistant animals, animals with increased growth rate can be obtained through genetic engineering. However, genetically engineered animals need to be carefully evaluated for food safety. In this evaluation process, the effects of genetic modifications on the health of animals, the environment and human health should be taken into account. Genetic engineering is a technology that brings both benefits and concerns about food safety. Potential benefits include more efficient food production, disease-resistant plants and animals, and improved nutritional value, while concerns include potential health effects, environmental impacts, ethical issues and consumer acceptance. In terms

of food safety, genetic engineering applications need to be evaluated and regulated, risk analysis, risk communication and regulatory mechanisms need to be used effectively (Muzhinji and Ntuli 2021; Bauer- Panskus et al., 2020; Sendhil et al., 2022; Mackelprang and Lemaux, 2020; Wang et al., 2020).

Food fraud is a serious problem that threatens consumers' access to healthy and safe food. Rapid growth in the food industry, increased global trade, complex supply chains and technological advances have contributed to the proliferation of counterfeiting and food fraud. Therefore, effectively combating food fraud has become an important priority to protect the health and confidence of consumers. An effective step in the fight against food fraud is the development of detection and analysis methods. Emerging technologies are used to detect signs of fraud and verify the origin of food products. Methods such as chemical analysis methods, DNA tracing, isotope analysis, spectroscopy techniques and molecular biology techniques help detect counterfeit or low-quality ingredients. In addition, advanced technologies such as big data analytics and artificial intelligence can be used to detect counterfeiting patterns and trace suspicious products. Cooperation and international standards are crucial in the fight against food fraud. Effective communication and cooperation between food inspection and regulatory bodies at national and international level is essential. In addition, a common framework for setting and implementing standards should be developed. International standards are a critical foundation for a more effective fight against food fraud. Consumer education and awareness is also crucial in the fight against food fraud. Consumers need to be made aware of the need to recognize counterfeit products and to exercise caution when shopping. Education programs and awareness campaigns can provide consumers with information on food fraud and encourage them to make the right choices. In addition, consumers should be informed about precautions such as buying from trusted suppliers, reading label information carefully and reporting suspicious cases to the authorities. Effective sanctions and criminal law measures are needed to combat food fraud. Deterrent penalties for those who produce or distribute counterfeit food can help reduce food fraud. Addressing regulatory gaps and implementing stricter sanctions can increase public confidence by ensuring that those who commit fraud are punished. Food fraud can be effectively tackled through strategies such as improved detection and analysis methods, cooperation and the establishment of international standards, education and awareness raising, sanctions and criminal law measures. The food industry, regulatory agencies and consumers working together will play an important role in reducing the problem of food fraud (Spink et al., 2019-1; Spink., 2019; Spink et al., 2019-2).

In recent years, research and innovation in the food industry has generated

great interest in artificial meat, laboratory-grown foods and new food sources. This field is considered as an alternative to traditional food production methods. Artificial meat is defined as meat products produced in the laboratory, while laboratory-grown foods are foods obtained by growing plant or animal cells in controlled environments. Novel food sources include potentially sustainable and nutritious sources that are different from traditional foods. Studies show that artificial meat, laboratory-grown foods and novel food sources have the potential to overcome some of the limitations of conventional food production. The potential advantages of these innovative approaches include reduced environmental impacts, increased efficiency of resource utilization, improved animal welfare and enhanced food safety. Artificial meat and lab-grown foods can produce fewer greenhouse gas emissions and make more efficient use of resources such as water and energy compared to conventional animal husbandry. At the same time, these approaches have the potential to improve food safety by reducing microorganism contamination.

New food sources have significant potential for diversity and sustainability. For example, foods produced using alternative sources such as seaweed, insects, algae and microorganisms can offer nutritious and environmentally friendly options. These sources also offer a different perspective on food safety. However, the implementation and acceptance of artificial meat, lab-grown foods and novel food sources face many challenges. Several factors must be taken into account for the successful development and utilization of these new technologies. Consumer acceptance, regulatory frameworks, technical and production processes, costs and ethical issues are among the key factors influencing the success of these innovative solutions. It is important that consumers trust and accept these new products, technological and production processes are optimized, and regulatory bodies establish appropriate guidelines and standards. Artificial meat, laboratory-grown foods and novel food sources are just a few of the innovative solutions that are expected to play an important role in food safety in the future. The development and application of these technologies represent important steps towards improving food safety, achieving sustainability goals and responding to consumer demands. However, alongside these issues, continuous work needs to be done, such as updating regulatory frameworks, continuing technological advances and raising consumer awareness. In this way, a safer, sustainable and diverse food system can be created in the future (Zhang et al., 2020; Bogueva and Marinova, 2020 ; Fraeye et al., 2020; Onwezen et al., 2021; Bryant and Barnett, 2020).

RESULTS

Contemporary Approaches to Food Safety: Risks, Innovations and Future Perspectives" covers a range of important topics in food safety, starting from basic concepts, such as risk assessment, prevention of foodborne diseases, innovative food processing techniques, technological innovations and future perspectives. It aims to establish a strong interconnection by bringing together important topics in food safety. Food safety is the effort to ensure that consumers have access to healthy and safe food. This chapter starts by emphasizing the importance of food safety and provides an overview of risk assessment, risk communication and risk factors. Prevention of foodborne illness includes sub-topics such as microbial pathogens, food poisoning and hygiene practices. In this way, understanding the risks to food safety and taking preventive measures are emphasized. At the same time, the importance of innovative food processing techniques is emphasized. Techniques such as high-pressure processing, ultraviolet irradiation, ozonation and electron beam ensure the safe processing and storage of food. In addition, there are advances in technological innovations such as rapid detection methods, biosensors, nanotechnology and artificial intelligence. These developments offer more effective solutions for food safety and increase the traceability of the food supply chain.

Future food safety perspectives are also addressed in this study. Topics such as genetic engineering, anti-counterfeiting, laboratory-grown foods and new food sources are areas that will have an impact on food safety in the future. Research and technological developments in these areas are enabling safer and more sustainable food production. These studies identify the issues that need to be focused on in the field of food safety and contribute to the development of future solutions. As a result, this volume provides up-to-date information in the field of food safety as well as an important perspective on future developments. Understanding food safety risks, evaluating innovative solutions and exploring future perspectives will help us take important steps towards access to safe and healthy food. This chapter is a valuable resource for researchers, academics, food industry professionals and anyone interested in food safety, and aims to raise awareness of food safety and encourage new approaches. We hope that future studies, innovations and solutions will contribute to safe and sustainable food systems.

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Chapter 13

The Negative Effects Of Excessive Irrigation On Lake Ecosystem

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ABSTRACT

Recently, agricultural areas are irrigated excessively by the fresh waters of the lake and small dams constructed on streams flowing into the lake, and free aquifers water obtained from the dejection cones and fans in the vicinity of lakes. The decrease of the lake level with the excessive irrigation has contributed to emerge of the shallow areas around the lakes, due to lowering of groundwater level. Some lakes like Lake Akşehir have completely dried up in the Central Anatolia. On the other hand, the drop of the lake levels has resulted in deteriorate of flora and fauna life in the lake biota and the population of native and migratory birds living in the swamps of the lake has decreased. The decrease in bird population has increased the insect pest population in agricultural areas and led to a decrease in fertility of agricultural products. The evaporation of saline and sodic lake waters in shallow areas caused by the accumulation of salts and alkaline substances rising on the soil surface by capillary. Dust blow containing salt and alkaline materials by the winds have resulted in the increase of respiratory disease in humans. As a result, excessive irrigation of agricultural lands has caused the environmental problems by damaging the lake ecosystem. In this study, negative effects in the regression of lake levels and emerged shallow lake areas and deterioration of the lake ecosystem depending on excessive irrigation will be discussed especially around the Lake Burdur.

Keywords: (Burdur lake, irrigated excessively, lake ecosystem,, wetland)

INTRODUCTION

The increasing need for irrigation water in the semi-arid regions of the world, especially in Sudan, Chad, Niger, Mali, Senegal in Sub Sahara Africa, and Iran, generally small agricultural lands are irrigated with groundwater and fresh water of some lakes. The places where the lake level drops in the lakes turn into different environments and soils according to the characteristics of the lake water. For example, hydromorphic and hydromorphic alluvial soils are found along the shore of freshwater lakes, and halomorphic soils appear in areas of salt and brackish water lakes. The decrease in the water feeding the lakes due to excessive irrigation has disrupted the lake ecosystem by upsetting the natural fauna and flora of the lake. All these desiccations and deteriorations have led to the deterioration of the ecosystem and human health.

After many lakes in the world dried up, epidemic diseases started and increased in the people living around it, and these diseases were respiratory problems, reproductuvite system problems, kidney and urological diseases, cancers, anemia and diarria (Sadeghi et al., 2019). In Aral and Urmiye lakes, which are the two largest and fastest drying lakes in the world, It has been stated that drying accelerated due to human influence and dust storms increased as a result of drying, and pollutants in the Aral lake caused serious health problems in the dry lake bed (Tussupova et al., 2020). The eastern Juyan lake in China decreased from 80 km² to 40 km² between 1960-1990 and completely dried up in the 1990s (Xiao et al., 2016). It has been determined that this drying was caused by the effect of climate change, but mostly by human activities, and stated that very serious regulations were made in government policies for the improvement of the lake. The water levels in Poyang and Dongting lakes decreased, the mud layer increased due to the impoundment in the Three Gorges dam, global climate change and sand mining, and consequently the migratory birds of Asia and the fish of the Yangtze river were affected (Wu et al., 2017).

Turkey is a country that connects the continents of Asia and Europe, is surrounded by seas, and has many lake and river ecosystems. The increase of temperature and decrease of rainfall in some part of Turkey have affected the ecological balance of the ecosystems. The semi-arid climate prevailing in the areas outside the Black Sea coastal region in Turkey the dry farming system has applied throughout the country. In order to get more agricultural product from agricultural lands, since the 1950s dry farming agricultural practice has been switched to irrigate agriculture. The use of water that is found in tectonic, landslide, karstic, alluvial barrier and volcanic lakes in Anatolia as drinking and irrigation water has caused to decrease of the lakes level.

It has been determined that after the Avlan lake in Antalya province was dried by state decision to gain land, drying and diseases started in the world's most valuable cedar forests around the lake, there were serious decreases in apple wheat and sugar beet yields, and the local people migrated from the countryside to the big cities. The west of Eğirdir, Turkey's second largest freshwater lake, has turned into land due to the lake's regression. The receding areas are currently the main formation areas of hydromorphic soils. However, it has been revealed that the lands that emerged as a result of the drying of the lake could not be used in plant production due to the fact that they are not young and qualified yet (Altunbaş, 2005). The flow resistance in Pupa, Hoyran and Gelendost streams pouring into Eğirdir Lake and associated it with precipitation (Tağıl and Alevkayalı, 2014). As a result of the research, a decrease was determined in the rivers and it was concluded that this decrease would affect the lake ecosystem and its environment in the long run. Konya basin, located in the south of Central Anatolia, *spagnum*, *tamarisk*, reed and so on. It contains many shallow lakes where hydrophytic plants grow. Recently, most of the shallow lakes have dried up due to excess water used for agricultural irrigation. Fauna, especially migratory and native birds migrated and some animals such as rabbits died. The excessive use of groundwater has led to the formation of small cylindrical karst depressions that have collapsed.

Eber Lake, which is one of the main reedy areas of Turkey such as *Phragmites* species, has turned into a semi-swampy land due to the dam built on the Çay Stream pouring into the lake. Thus, the areas of formation of hydrophytic vegetation in Lake Eber have been significantly reduced. The people of Eber village, who make their living from reed production, became unemployed and fish production decreased. As a result of the significant decrease in the water flowing from Eber Lake to Akşehir Lake and the excessive use of this water in the irrigation of agricultural areas, Akşehir Lake has completely dried up. Thus, the people engaged in fishing in Lake Akşehir became unemployed. Native and migratory bird populations have migrated to other regions. Marmara Lake, which is an alluvial depression set lake in the north of the Gediz Graben in the Aegean Region, has reduced the lake area and the depth of the lake in irrigation of agricultural lands. This situation brought the fishing in the lake to a standstill.

This study focused on the causes and consequences of the drying up of the Burdur lake, located in the south of Turkey. In the field surveys, the water supply sources of Burdur Lake, the physical and chemical properties of the soil formed in the lake areas, and the negative effects of excessive irrigation applications on vegetation and flora life were investigated.

2. MATERIALS AND METHODS

2.1. Material : Burdur Lake is a tectonic depression lake located in the Burdur closed basin, within the scope of the Ramsar wetland, on the borders of Burdur and Isparta provinces in the south of Turkey. (Figure 1).

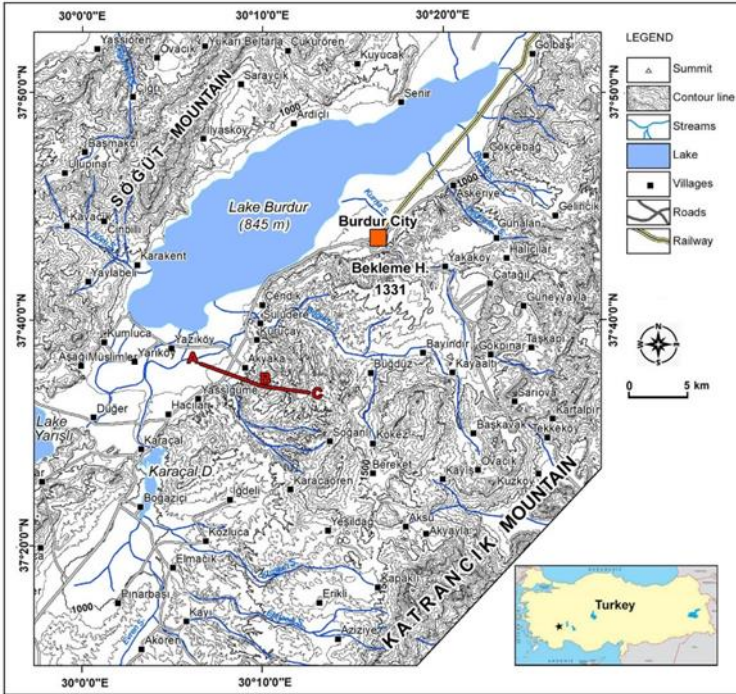


Figure 1. Location and topographic map of Burdur Basin

Climatic type of the study area is Mediterranean semiarid transitional climate between Mediterranean and continental Central Anatolia. Mean annual temperature which is 13°C in the lowland decreases some 6-7°C on the upper part of the mountains. January temperature which is the 2-3°C on the lowlands falls under freezing point. Mean annual precipitation is about 430 mm, most of which fall between December and April, and fluctuates between 270 and 600 mm (Atalay et al., 2019).

There are close relationship between the geomorphic units that have formed in the different geological period and parent materials and main soil types in the Burdur-Göhlhisar Basin (Altunbaş, 2018). There are rocks belonging to different geological periods around the Burdur Lake. There are Neocene marl in the east and southeast of the lake, silica cemented sandstone in the northeast, sandstone and conglomerate in the northwest, limestone and serpentines in the southwest (Atalay et al., 2019). According to soil classification of Turkey the soil

developed on the marl deposit under the xerophytes vegetation and semiarid climates was classified as Rendzic Leptosols (Kurucu et al., 2018).

According to Kesici and Balpınar *Phragmites australis*, *Typha domingensis* are found around the lake, *Suaeda cucullata*, *Salicornia euroaea*, *Salsola kali*, *Petrosimonia brachiata*, *Ailanthus altissima* around the lake, *Tamarix sp* in sandy areas, *Cardopatum corymbosum* and *Juncus maritimus* in the old retreat areas of the lake (on the barren sediments where the lake dries up). Algae, zooplankton, phytoplankton, invertebrates, Burdur moss fish *Aphanius sureyanus*, frogs, turtles and reptiles around the lake, and about 454 bird species such as vertical duck, grebe, Hungarian duck, flamingo and crane live in Burdur Lake (.Gülle and Atayeter, 2016)

2.2. Methods: Topographic maps in the scale of 1/25 000 and modified geologic map in the scale of 1/100 000 were also used. The samples of the soil and semi weathered parent materials of the experimental sites were taken to determine of texture, pH, EC, CaCO₃, organic matter and CEC (Cation exchange capacity). E.C. (Exchangeable Cations) their determinations were made at the Soil Laboratory of Soil Science and Plant Nutrition of Agricultural Faculty, Akdeniz University. Texture of soil samples determined based on Bouyoucos hydrometer method (Bouyoucos, 1955) and organic material was made Walkley-Black (Black, 1965), CaCO₃, EC and pH determined (Jackson, 1967), Exchangeable Cations determined according to 1N ammonium acetate method (Kacar, 1995). Cation Exchange Capacity (CEC) determined According to sodium acetate method (Kacar, 1995), respectively. Cl (Johson and Ulrich, 1959), SO₄ (Fox et al., 1964), CO₃ and HCO₃ (Ayyıldız, 1994) was made.

3. RESULTS AND DISCUSSION

There are more than 100 lakes of tectonic, tectonic-karstic, karstic, volcanic, landslide and barrier origin in Turkey. While there are salty and brackish water lakes in closed lakes, fresh water is dominant in open basin lakes feeding streams. As it is known, lakes are not only an area where water is collected, but also important sources that provide drinking and utility water for settlements that irrigate the surrounding agricultural areas. Protecting the lake ecosystem means continuing sustainable agriculture, animal husbandry and fishing activities.

In this study, which was carried out on the soils around Burdur Lake, the use of water in agricultural lands and its effects on the soil and lake ecosystem were investigated. The water needed for agricultural activities around the lake is

mostly produced by illegal and official wells. Due to the excessive use of groundwater, the amount of water reaching the lake has decreased somewhat, which has reduced the water volume of the lake. On the other hand, dams were built on some small streams pouring into the lake. Thus, the shallow areas in the northeastern part of the lake dried up and emerged into the terrestrial environment.

Burdur Lake does not have the characteristics of drinking and irrigation water in terms of water quality. The water of Burdur Lake, which contains Na/Mg/Cl/SO₄/HCO₃, shows a high alkaline reaction (pH>9). Due to its C5S5 class, it is not used for drinking, irrigation, industry and agriculture purposes. The salt content is more than 200‰. Its electrical conductivity was measured as 31.7 mS/cm (Gülle and Atayeter, 2016). For this reason, the surface and underground waters carried into the lake are used excessively before they reach the lake.

Various physical and chemical analyzes were carried out on soil samples taken from the northeast of the lake (Table 1). According to the results of the analysis, a very thin A horizon was found on the lacustrine materials released to the terrestrial environment, and A/ Cg horizon that could not develop a structure just below it. Soil formation is negligible. Soils are soils with high pH, salty, alkaline, very calcareous, insufficient organic matter, silty clay-clay (SiC-C) texture, high sulfate and chlorine content, and significantly high Na content in exchangeable cations.

Table 1. Some physical and chemical properties of the youngest soils of Burdur lake

	pH	EC dS/m	CaCO ₃ %	O.M %	Texture	CEC me%100g	E.C. me/100g		Anions				
							Na	K	Ca+Mg	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻²
A	7.95	17.9	20.0	2.5	SiC-C	55.3	35.7	0.6	22.5	0.7	2.5	72.4	28.4
Cg	7.95	20.0	21.8	2.7	C	57.4	32.5	1.0	24.0	0.2	0.9	66.1	14.4
A1	7.62	16.8	45.0	0.85	SiC	54.9	35.6	0.8	27.2	0.3	1.0	68.8	21.9
A2	7.73	16.8	22.1	1.94	C	58.3	35.5	0.8	23.7	0.2	1.2	70.1	21.9
A	7.62	17.9	22.0	0.95	C	58.5	36.4	0.8	21.6	0.5	0.9	71.6	21.9
C	7.90	18.3	23.7	0.50	C	58.5	33.5	0.8	22.1	0.4	0.8	72.6	21.9

The altitude of Burdur Lake, which was measured as 860 m in 1950, decreased to 842 m in 2020. The water surface area emerged with the 30% decrease in the lake level. This decrease in the level of Burdur Lake has caused the expansion of the swampy land near the lake shore and the increase in sodium, calcium and salty minerals on the marl bed in the lake area, which regressed due to capillary. The white color on the hydromorphic soil surface in

the north of the lake indicates that sodium, magnesium and calcium compounds accumulate as a result of capacity and evaporation (Figure 2, 3).



Figure 2. The concentration of salt and limely material nodules on the emerged lake area in the northeastern part of Lake Burdur

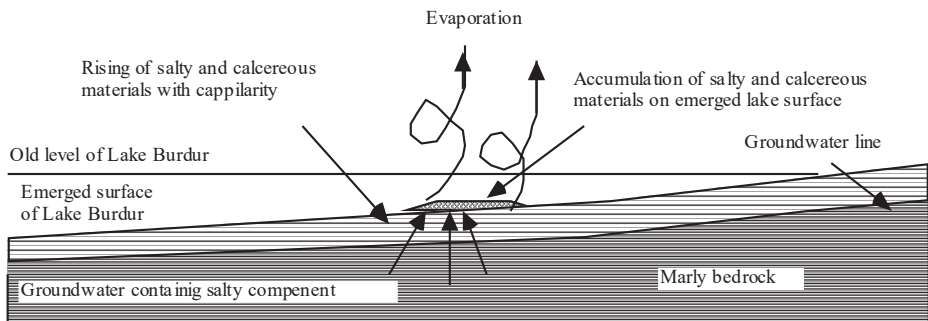


Figure 3. The rising of salty, calcereous material material to be found in the lake water and marly parent material/ bedrock with cappilarity process and the formation of the accumulation of salty and calcereous materials on the emerged Burdur Lake surface (Atalay, et al., 2019)

As a matter of fact, white nodules on soil surfaces contain 10 000 ppm calcium, 3000 ppm magnesium and 8000 ppm sodium. The transport of salt and alkali substances by wind caused air pollution. This air pollution will affect not only human health, but also the meat and milk quality of animals grazing in this area (Atalay, et al., 2019). According to (Gözükara et al., 2019), drying in the northeastern part of the lake is more intense in the southwestern part, and it shrank by 38.34% between 1975 and 2017 (Figure4).

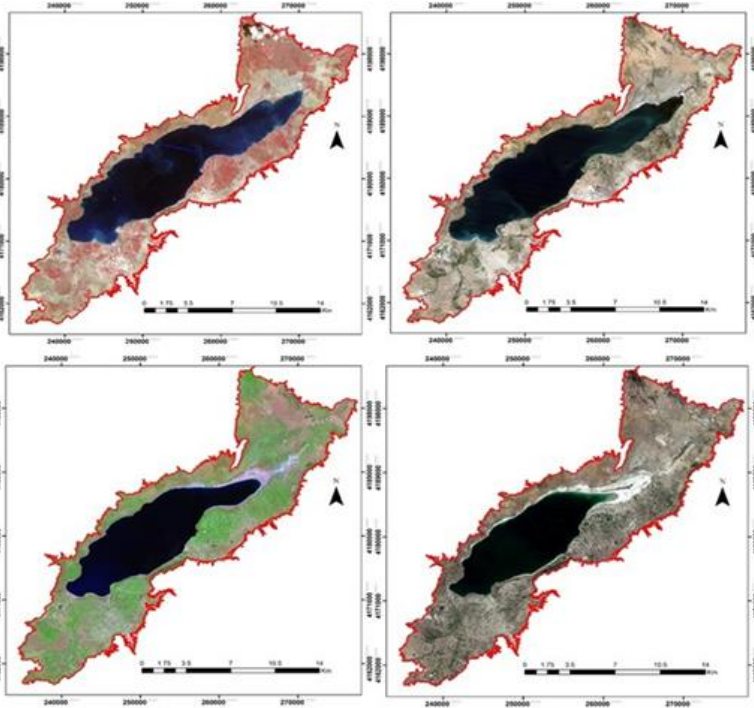


Fig 4. Satallite images of 1975 (a), 1987 (b), 2002 (c), 2017 (d) (Gözükara 2019).

Another problem relating to the drop of the lake level is to encourage the fluvial backward erosion occurring in the lower watershed area of the streams. In order to partly mitigate the problem taking place in surrounding areas of Lake Burdur it is necessary to prevent excessive pumping of ground water and to lessen the dam construction on the streams (Atalay et al..2019)

4. CONCLUSIONS

It is clearly seen that Burdur Lake is drying up at a great speed. Other studies in the field have revealed that this drying is not only global climate change, but also excessive use of groundwater feeding the lake and the construction of many dams in front of its streams. As a result of the evaluations, the problems and solution proposals regarding the drying up of Burdur Lake are given below.

1. Over-irrigation of agricultural lands around the lake by illegal wells is a problem. The opening of new wells should be prevented legally and technically and the local people should be made aware of this issue.

2. Started dam constructions should be completed and new dam construction should be prevented. If needed, some dams have to re-run the water into the lake to slow drying out.

3. Terrestrial areas formed on the shore of the lake have led to the formation of biomes as well as hydrobiomes and halobiomes, depending on the chemical and lithological properties of the lake water and parent material. Namely, salty lake water. The coastal zone where it emerged has been transformed into a halobiome characterized by saline soil, and freshwater areas have been replaced by predominantly hydromorphic soils. These soils should be used in accordance with their capabilities.

4. Na, Ca, Mg and K, which are mixed into the air as dust from the dried surfaces, threaten the settlements in the east of the lake. New measures should be taken to prevent the people and other creatures living in this region from being affected.

5. The decrease in the water resources feeding the lake has disrupted the lake ecosystem. Studies and trainings should be carried out for the rehabilitation of the ecosystem in and around the lake.

6. Drip irrigation should be used in agricultural lands, water savings should be made in residential areas and recreational areas.

7. Drought resistant crop should be cultivated.

Author Contributions: The following supporting information can be downloaded at Figure 3: Ministry of Agriculture and Forestry Publication: ISBN 978-605-7599-16-2. Ecology, Ecosystem, Habitats and Rehabilitation of Marble Quarries of Burdur Gölhisar Basin, NW Anatolia. P:84; Figure 4: Temporal and Spatial Change the Lacustrine Material Formed as a Result of the Level Change in Burdur Lake. Anadolu Journal of Agricultural Sciences 34(2019), doi: 10.7161/omouanajas. 556215, p: 389.

Table 1: Punto ve Yazı Tipi Özellikleri (Times New Roman)

Konu	Punto	Yazı Tipi Özelliği
Başlık	14	Kalın
Yazar bilgisi	10	Normal
Abstract, keywords, JEL classification	11	Normal
Özet, anahtar kelimeler, JEL kodu	11	Normal
Metin içi yazı	11	Normal
Birinci Seviye Başlık	11	Büyük Harf, kalın
Alt Başlıklar	11	Küçük harf (ilk harf büyük), İtalik, kalın
Tablo ve Şekil İsimleri	11	Normal
Tablo verisi ve kaynak	9-11	Normal
Referanslar	10	Normal

Source: Tablo, şekil vb. alıntı ise altında kaynak olmalıdır.

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Chapter 14

Speed Breeding: An Innovative Approach for Accelerated Genetic Improvement in Agricultural Crops and Integrating with Molecular-Based Approaches

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ABSTRACT

Speed breeding techniques utilize controlled environmental conditions where various manipulation methods are employed to regulate light duration, intensity and temperature. Artificial environments for plant growth were first introduced by botanists in the 1980s. NASA and Utah State University researchers explored the effects of constant light on accelerated plant growth cycles, leading to the development of USU-Apogee, a dwarf wheat variety. Dr. Lee Hickey and his team at the University of Queensland refined the approach in 2016 to expedite plant production and improve breeding efficiency.

One of the major benefits of speed breeding is its capacity to swiftly incorporate specific traits into crops. These traits encompass disease resistance, pest tolerance, nutritional content and quality attributes. By utilizing methods such as genetic engineering or marker-assisted selection, desirable genetic traits can be integrated into crops more efficiently, hastening the development of new cultivars with improved characteristics. Furthermore, speed breeding can be combined with other strategies like pollen selection, genome editing and high-throughput phenotyping to enhance breeding programs' overall effectiveness. The integration of Marker Assisted Selection, speed breeding, genomic selection, and the CRISPR/Cas system enhances accurate selection, increases genetic gain, and addresses regulatory concerns related to genetically modified products. This approach accelerates plant breeding programs and selects desirable traits.

In conclusion, speed breeding technology has enormous potential to revolutionize crop improvement and meet the rising global food demand. It is time to invest in this technology and explore its full potential to create a more sustainable future for all.

Keywords: Speed breeding, CRISPR/Cas, MAS, Genomic Selection, genetic gain.

INTRODUCTION

Climate fluctuations such as droughts, floods and high temperatures along with the increase in global population, pose a serious threat to food security (Ray et. al., 2013). Many researchers have emphasized the importance of achieving genetic gains in agricultural crops more rapidly to meet global food demands (Lin et. al., 2016). Developing resilient and fertile varieties in a shorter period of time by using traditional approaches continues to be a challenging process for plant breeders (Samantara et. al., 2022). To overcome the disadvantages associated with traditional methods and maintain food security, the concepts of rapid breeding are now being adopted to achieve rapid genetic gains in various crop species.

Plant breeding is an art and science of modifying the genetic of plants to obtain desired traits in any given species. In this process, changes can be made to the genetic structure of plants through naturally occurring variations or mutations that can be utilized by breeders. The goal is to develop plants with improved tolerance to biotic and abiotic stresses, along with higher yield potential and nutritional quality (Breseghello and Coelho, 2013; Tester and Langridge, 2010).

Breeding new and improved varieties for most field crops takes years. (a) The selection of parent plants with desired complementary traits; (b) Hybridization of the selected parents using appropriate methods; (c) Selection and genetic advancement based on target traits; (d) Identification of the best-performing and stable candidate varieties; and (e) Variety registration, seed multiplication and distribution for production (Shimelis and Laing, 2012). This is a significant disadvantage, particularly for crops grown in the field with only 1-2 generations per year.

In the absence of an integrated pre-breeding program, developing and homogenizing a variety through traditional breeding procedures can take more than 10 years (Ahmar et. al., 2020; De La Fuente et. al., 2013). The time involved in these stages significantly delays varieties' development and commercialization. However, the rapid growth of modern agriculture and the increasing demand for food have necessitated the use of faster and more efficient technologies in plant breeding methods. In this context, a new technology called "speed breeding" has been developed.

Speed breeding is a technology used to accelerate the plant breeding process. This method involves a set of factors that enhance the growth rate of plants. These factors include high light intensity, increased carbon dioxide levels, optimized temperature and humidity conditions. The combination of these factors speeds up the growth process of plants, enabling faster and more efficient hybridization and selection processes in the breeding programs (Singh and Jeneja, 2021).

Speed breeding techniques utilize controlled environmental conditions where various manipulation methods are employed to regulate light duration, intensity and temperature. The concept of utilizing artificial environments for plant growth was first introduced by a group of botanists many years ago. In the 1980s, scientists from the National Aeronautics and Space Administration (NASA), with collaboration with Utah State University, adopted similar protocols to explore the effects of constant light on accelerated plant growth cycles. This research led to the development of a new dwarf wheat variety called USU-Apogee (Bugbee et. al., 1997). Building upon this work, Dr. Lee Hickey and his team at the University of Queensland in Australia further refined the approach in 2016 to expedite plant production and enhance the efficiency of the plant breeding process.

The speed breeding method facilitates an accelerated reproductive process in plants, enabling the rapid development of new varieties. Its initial application focused on investigating seed dormancy in *Triticum aestivum* (bread wheat) under controlled conditions (Hickey et. al., 2009). Over the years, speed breeding protocols have been successfully employed in various crops such as oats (Liu et. al., 2016), barley (Hickey et. al., 2017), wheat (Mukade, 1974), chickpea (Watson et. al., 2018), lentils and peas (Mobini et. al., 2015), allowing for advancements of approximately six generations per year. This expedited breeding approach holds great promise for accelerating the development of improved plant varieties.

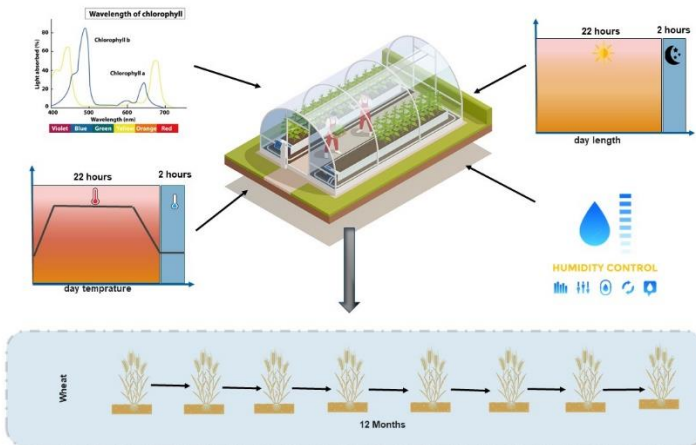


Figure 1. Rapid generation advancement through speed breeding: Experimental crop grown under controlled environments.

A few selection techniques that can be used in speed breeding to shorten the breeding cycle are single seed descent (SSD), single pod descent (SPD), single plant selection (SPS), clonal selection and marker-assisted selection (MAS)

(Samineni et al., 2019; Watson et. al., 2018; Hickey et. al., 2017). Speed breeding can yield 3 to 9 generations per year, whereas traditional selection processes typically produce 1 to 2 generations per year (Ghosh et. al., 2018; Ochatt et. al., 2002). Speed breeding permits the quick establishment of homozygous and stable genotypes, speeding up generational development and the creation and dissemination of novel cultivars (Watson et. al., 2018). Additionally, MAS and fast breeding technologies work well together. (Samineni et al., 2019; Watson et. al., 2018; Hickey et. al., 2017).

In addition to adapting to their specific habitats, plants have developed requirements for the quantity and quality of light. Because of this, plants are divided into long-day and short-day plants based on how much light they require (Samantara et. al., 2022). Continuous lighting shortens the vegetative phase of long-day plants and speeds up the reproductive cycle, allowing scientists to collect more generations annually. In contrast, short-day plants need a certain illumination scheme in order to advance quickly to the reproductive stage (Sysoeva et. al., 2010). Each plant species' unique needs for day length, which directly influence the length of the flowering season, determine the methodology for this technique (Samantara et. al., 2022). By adjusting the light intensity and duration, the speed breeding technique also takes into account other factors influencing plant development, such as temperature requirements, humidity and soil composition, which vary with each crop (Jähne et al., 2020).

SPEED BREEDING FACTORS

Light

The most important factor in speed breeding is the light source. In the growth chamber, artificial light is produced by using LED, halogen, high-pressure sodium (HPS) or metal halide (MH) bulbs that supply plants photo-synthetically active radiation (PAR) for photosynthesis. For each crop, the temperature and humidity are different (Velez-Ramirez et. al., 2011). By using light-based speed breeding procedures, the breeding strategy with photosensitive crops can be designed effectively. The rate of photosynthesis, gas exchange, transpiration, stomatal activity and other plant growth activities are all significantly influenced by the quality of light given every single day (Yang et. al., 2017). Many lighting sources are appropriate for speed breeding, including SVLs and LEDs. However, the choosing process should take into account the plant species, available area, and energy sources. For instance, basic incandescent lighting may be appropriate in a smaller space with adequate cooling to offset the increased heat production, but LED lighting may be chosen due to its energy efficiency (Sreya et. al., 2018).

The application of 360–650 $\mu\text{mol}/\text{m}^2$ light intensity within the 400–700 nm PAR (photosynthetically active radiation) range has been found successful for promoting early flowering and seed formation in barley, wheat, chickpea, canola and other important crops. The use of blue and far-red light spectra has been observed to induce early flowering in legumes such as chickpea, pea and lentil (Mobini et. al., 2015). A fundamental requirement is approximately 22 hours of photoperiod with 2 hours of darkness. Additionally, different plant species may respond differently to the spectra of wavelengths emitted by various lighting sources so this factor should be carefully considered in the application of speed breeding techniques.

Temperature

In cultivated plants, the change from the vegetative stage to the flowering stage is greatly influenced by temperature. It has an impact on the rate of seed germination, plant growth, flowering time and maturity. According to Hatfield and Prueger (2015) and McClung et. al. (2016), very low and high temperatures have a wide range of consequences on plant development, including the change from vegetative to reproductive phases. Most crops require temperatures between 12 to 30 °C for germination and 25 to 30 °C for growth, flowering and seed set (Dubcovsky et. al., 2006). However, certain crops require temperatures as low as 12 °C for germination and as high as 30 °C for growth. The reactions of photoperiod-sensitive genotypes of different crops to temperature regimes that influence their transition from the vegetative to the reproductive stage have shown variation (Yang et. al., 2014).

Soil moisture

Plant growth and development processes, such as plant height, flowering period, seed production and maturity, can be strongly impacted by variations in soil moisture (Hussain et. al., 2018; Anjum et. al., 2017). Both drought and flooding stress can be used to promote early flowering and expedite maturity in speed breeding. Soil moisture stress plays a critical role in affecting plant growth and development. While managed irrigation during wilt symptoms can improve plant growth and development, drought stress is frequently applied to crops like wheat, barley and pearl millet (Shavrukov et. al., 2017; Zheng et. al., 2013). These methods improve crop water use, allowing for a faster generational cycle in speed breeding (Hussain et. al., 2018).

For most plants, maintaining a humidity level of 60–70% is generally regarded as optimal. Many crop species demonstrate early blooming and seed production when under moisture stress conditions (Shavrukov et. al., 2017). Rapid

generation and development of crop species can be facilitated by adjusting soil moisture conditions throughout the speed breeding process. In a variety of crops, including wheat, barley, canola and chickpea, a controlled decrease in soil moisture levels near the end of the flowering stage encourages considerable grain filling and maturation (Watson et al., 2018).

CO₂ levels

Crop plants have been reported to undergo a shift from the vegetative to reproductive phase in response to increased levels of carbon dioxide (CO₂). Elevated CO₂ levels can promote plant growth and accelerate the transition from vegetative to reproductive stages, although the response may vary among different crop species and even within genotypes of the same species (Jagadish et. al., 2016). In soybean, rice and cowpea, higher CO₂ levels have been found to reduce flowering time, while in soybean, maintaining CO₂ at a low level has been shown to delay flowering time (Sreeharsha et. al., 2015; Bunce, 2015; Springer and Ward, 2007). Furthermore, the combination of CO₂ supplementation with specific light/dark cycles has been observed to shorten the crop cycle and increase the number of generations per year in soybean (Nagatoshi and Fujita, 2019).

Plant density

Utilizing high-density planting is an economical approach in speed breeding, as it facilitates rapid generation turnover while maintaining a substantial population size. This method involves growing crops at higher densities, which promotes early flowering and maturity, ultimately increasing the number of generation cycles per year (Warnasooriya and Brutnell, 2014). The effectiveness of high-density planting can vary depending on the specific plant species and genotype. For instance, in rice, employing a high-density planting of 400 plants/m² allowed for up to four generations per year, reducing the crop cycle length by 15 to 40 days (Rahman et. al., 2019). However, the impact of high-density planting on flowering is not consistently observed across studies, and genotype variations significantly influence plant responses (Fukushima et. al., 2011; Hayashi et. al., 2006).

Sorghum has exhibited diverse responses to high-density planting (Villar et. al., 1989), while in cotton, high-density planting (11 plants/m²) has been found to shorten the blooming period to 25–26 days compared to lower density planting (9 plants/m²) (Khan et. al., 2017, 2019). On the other hand, some researchers have observed no significant differences in the onset of blooming with high-density planting (Fukushima et. al., 2011). Therefore, it is important to investigate the genotypic responses within each species to determine the feasibility of high-

density planting as a component of speed breeding. Preliminary trials are necessary to identify the specific high-density planting requirements for a given genotype and optimize early flowering. High-density planting serves as a cost-effective strategy in speed breeding, allowing for rapid advancement of generations while maintaining the large population sizes required for advanced selections.

Plant growth regulators

Plant growth regulators have been employed to expedite growth, stimulate flowering, seed formation and enhance the germination of immature seeds in vitro conditions. The utilization of plant growth regulators within controlled environments like greenhouses and growth chambers, where photoperiod and temperatures can be meticulously regulated, can yield diverse outcomes (Bermejo et. al., 2016). For example, the combination of auxin and cytokinin hormones, specifically flurprimidol, indole-3-acetic acid and zeatin, has been shown to promote in vitro flowering and seed formation in faba bean (Mobini et. al., 2015). Additionally, the exogenous application of 6-benzylaminopurine has demonstrated increased seed set in faba bean (Mobini et. al., 2020). In lentils, a combination of flurprimidol and 4-chloroindole-3-acetic acid has led to flowering and seed formation, while adjusting the photoperiod, temperatures and administering plant growth regulators has reduced the generation cycle in both lentils and faba beans, enabling the achievement of up to 8 generations per year (Mobini and Warkentin, 2016). Embryonic seed culture using immature embryos cultivated on a medium known as MS culture medium has proven suitable for lentil embryo culture (Bermejo et. al., 2016). In the case of wheat, embryo culture on half-strength MS medium supplemented with potassium dihydrogen phosphate and sucrose has induced flowering and seed set (Yao et. al., 2016). Drying and chilling methods have also been employed to break seed dormancy in immature seeds of various crops, including wheat and barley, resulting in high germination rates (Watson et. al., 2018). The utilization of immature seeds and optimal germination conditions can be applied both in field and indoor settings to accelerate generation advancement (Ghosh et. al., 2018; Saxena et. al., 2017).

SPEED BREEDING: ADVANTAGES, CHALLENGES, COMPARISONS AND INTEGRATING

Advantages

Speed breeding refers to a collection of techniques aimed at expediting the flowering and seed formation of crop genotypes by manipulating their growing environment. This approach allows for rapid progression to the next breeding

generation, saving time and resources (Wanga et. al., 2021). Unlike the traditional cycle of 2 to 3 generations per year, speed breeding enables the production of 3 to 9 generations annually for crops like wheat, barley, and chickpea (Ghosh et. al., 2018; Ochatt et. al., 2002). One of the major benefits of speed breeding is its capacity to swiftly incorporate specific traits into crops. These traits encompass disease resistance, pest tolerance, nutritional content and quality attributes. By utilizing methods such as genetic engineering or marker-assisted selection, desirable genetic traits can be integrated into crops more efficiently, hastening the development of new cultivars with improved characteristics (Hickey et al., 2019).

In addition to its time and resource-saving advantages, speed breeding empowers plant breeders to promptly respond to changing environmental conditions and emerging plant diseases. By employing speed breeding, breeders can swiftly generate new plant varieties that are better adapted to environmental challenges like drought, heat or salinity (Sharma et. al., 2022; Begna et. al., 2022). This capability helps farmers maintain crop yields and ensure food security despite evolving environmental conditions and emerging plant diseases. Moreover, speed breeding reduces reliance on chemical inputs such as pesticides and fertilizers by developing crop varieties that exhibit enhanced resistance to pests and diseases, as well as higher nutrient efficiency. Ultimately, speed breeding saves the time and cost associated with developing and testing new crop varieties, leading to more efficient and accelerated plant breeding programs (Xu et al., 2022; Bonea, D., 2022).

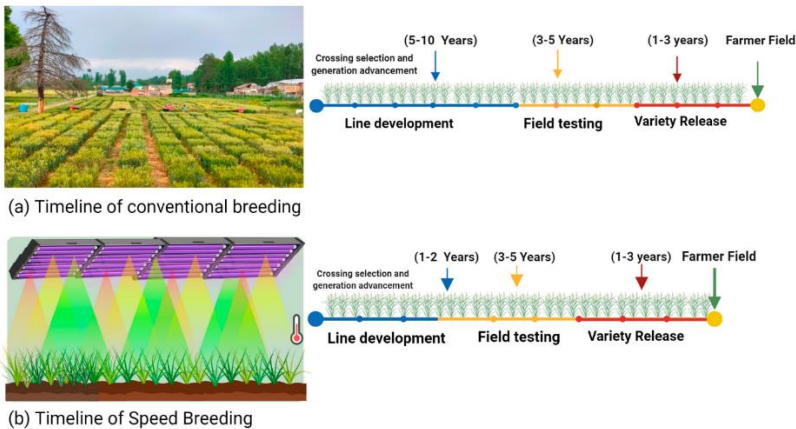


Figure 2. Comparison of traditional breeding and speed breeding (Samantara et. al., 2022).

Challenges of speed breeding

For various plant species, the speed breeding method offers both unique challenges and advantages. Under prolonged photoperiods, the time to flowering is accelerated in plants with long-day and day-neutral responses. Speed breeding conditions may not be suitable with the shorter photoperiods in short-day plants (Thomas and Vince-Prue, 1996). The speed breeding strategies of each species can be enhanced by experimenting and optimizing light and temperature factors. Even though speed breeding procedures take place in a lab, they can be helpful for tasks like crossing, SSD and simple trait screening. Although selection for adaptation to the target environment must still place in the field (Watson et al. 2018).

Programs that alternate between field selection and speed breeding can expedite the process overall. Immature seed harvesting can be used to speed up generation times even more, but it may interfere with the phenotyping of some seed traits. For instance, under speed breeding conditions, the phenotyping of seed dormancy in spring wheat breeding programs is restricted to just four generations per year (Hickey et. al., 2009).

Building a growth chamber or glasshouse with the right lighting and temperature control capabilities can require a sizable initial investment, but the advantages might outweigh the costs. To reduce operating costs, it is also important to choose lighting and temperature control systems carefully. Comparing supplemental LED lighting to other lighting options like SVLs, supplemental LED lighting is a more affordable and energy-efficient choice (Collard et. al., 2017). Although solar panel use can reduce costs, a cost-benefit analysis should be done to determine whether speed breeding programs are feasible.

Overall, considering the potential advantages for variety development, research output is crucial when incorporating speed breeding into a crop improvement program. Different breeding programs that use speed breeding can be evaluated using computer simulations and the cost of speed breeding should be compared to any potential long-term rewards.

Comparison of speed breeding with other breeding techniques

There are several strategies available to enhance generation turnover in plants, including shuttle breeding, photoperiod manipulation, physiological stress and embryo rescue. Shuttle breeding, pioneered by Norman Borlaug, involves planting wheat populations in different field locations with varying altitudes, latitudes and climates to achieve two generations per year (Ortiz et. al., 2017). Inducing physiological stress, such as limiting plant growth area, restricting

nutrient and water access and providing intense light, can trigger early flowering and seed development in numerous species (Wada and Takeno, 2010). Embryo rescue, a technique that stimulates germination of immature seeds on a culture medium, eliminates the waiting period for seed maturation and has been successfully employed, with or without the use of plant growth regulators to achieve multiple generations per year in lentils, fava beans, subterranean clover, wheat, barley, peas, and soybeans (Mobini et. al., 2015).

The generation time can be further shortened by combining embryo rescue with other methods like applying stress and using plant growth regulators. For instance, under shuttle breeding conditions, wheat and barley seeds can be harvested early, just two weeks after pollination and then quickly dried and chilled to achieve high and uniform germination rates, leading to healthy plants. If the right circumstances and resources are present, these techniques show promise for enhancing generation turnover in other cereal, pulse and legume crops and can greatly aid breeding and research programs (Velez-Ramirez et. al., 2014; Gebologlu et. al., 2011).

Researchers and breeders are attempting to create novel breeding procedures that can shorten breeding cycles and lower the number of cycles needed to create new varieties in order to overcome breeding difficulties (Khoury et. al., 2014). Breeding cycles have already been shortened and genetic gain rates have increased in crops like wheat, rice and maize thanks to recent advancements in breeding techniques, including phenotyping and genotyping, marker-assisted selection, genomic selection and CRISPR gene editing (Watson et. al., 2018). The impact can be considerably larger when these technologies are combined with speed breeding methods, which cultivate plant populations under controlled photoperiod and temperature regimes to hasten their growth and development. With the difficulties facing global food and nutrition security, speed breeding can help to quickly increase generation turnover and shorten the time needed to generate new kinds (Hickey et. al., 2017).

Integrating Marker-Assisted Selection, Genomic Selection and CRISPR/Cas System for Genetic Gain and Accelerated Trait Development

The productivity of agriculture has improved in developed nations, but this has put more strain on the food production industry. Plant breeding can be used to create plants with specific traits and current scientific advancements offer a variety of opportunities and breakthroughs in this field (Varshney et. al., 2006). The existing rate of annual yield enhancement in the principal crop species must be quadrupled to meet the steadily rising demand for plant-based products (Varshney et. al., 2006).

Crop breeding has seen a radical transformation over the past 150 years due to advances in genomics and molecular techniques (Collins et. al., 2003), with strategies like genomic selection, phenotyping and speed breeding. To enhance the breeding of commercially significant crop species, methods like genetic engineering, molecular markers and large-scale sequencing have been proposed (Majid et. al., 2017). Since the 1990s, superior hybrid lines have been selected by using molecular markers and breeders frequently concentrate on diploid or diploid-like crops with quicker reproductive cycles. Genome research and plant breeding improve breeding procedures' precision and speed up the process (Zhang et. al., 2014); Collins et. al., 2003).

Marker Assisted Selection (MAS) is a technique employed in plant breeding that utilizes DNA-linked markers to indirectly choose desirable traits. MAS has been extensively used in plant breeding and can expedite generation turnover in various crops (Das et. al., 2017; Prabhu et. al., 2009). MAS operates on the principle of identifying DNA markers closely associated with the gene of interest. These markers serve as indicators for the desired traits. By selecting plants based on these DNA markers, breeders can efficiently choose individuals with the desired characteristics. The subsequent stabilization of these traits can be achieved through speed breeding (SB). To enhance the efficiency of MAS at the individual plant level, techniques like "seed chipping" with barcoding can be employed. SB can be integrated with other breeding methods like backcrossing, pyramiding and transgenic pipelines to accelerate the overall breeding processes (Watson et al., 2018).

In contrast, Genomic Selection (GS) utilizes dense markers spanning the entire genome to estimate genomic breeding values (GEBVs) that capture the collective influence of multiple quantitative trait loci (QTLs). This comprehensive approach allows for the evaluation of genetic variance for complex traits like yield, which are influenced by numerous genes (Hayes et al., 2001). Plants with higher GEBVs are advanced to the subsequent generation. GS stands out from other breeding methods due to its efficiency in selecting varieties, saving time and resources in the breeding process. Notably, GS has been successfully applied at the industry level, such as in maize breeding for drought tolerance, leading to the release of "AQUAmax" hybrids for farmers' use (Cooper et. al., 2014; Gaffney et. al., 2015).

However, the primary challenge associated with GS is the cost of genome sequencing. To mitigate this concern, GS can be implemented in alternate generations or selectively utilized for specific traits that surpass a predefined threshold. In such cases, other breeding methods like rapid generation (SB) can

be employed to complement GS and reduce expenses (Riaz et al., 2016). This strategy helps optimize the utilization of GS in plant breeding programs.

Speed breeding (SB) and genomic selection (GS) can be combined to improve selection accuracy and accelerate genetic progress annually (Gorjanc et al., 2018; Hickey et al., 2017). This method involves choosing parents based on their genomic estimated breeding values (GEBVs), which significantly reduces the time needed for selection, and then using SB to produce the chosen progeny. Speed breeding cycles are made possible by this iterative process. Within a breeding program, the genetic gain can be further increased in each generation by combining SB and GS (Crain et. al., 2018). This combined strategy reduces the problems associated with inbreeding compared to phenotypic selection or a standalone GS scheme. Additionally, by utilizing cutting-edge high-throughput phenotyping platforms that allow for early-stage selection, it can produce greater gains when applied to multiple traits, such as in normalized difference vegetation index and canopy temperature (Crain et. al., 2018). In comparison to conventional selection and breeding techniques, simulation studies examining the combination of GS and SB for various traits with differing genetic architectures and heritabilities have revealed additional genetic gains and shorter generation times (Jighly et. al., 2019).

Due to their rapid reproduction and vast population size, plants are more applicable to genetic manipulation than other kingdoms. Speed breeding, a non-GMO technique (Voss-Fels et. al., 2019, Watson et. al., 2018), enables scientists to pick plants for desired features among variations after turning over many generations. (Watson et. al., 2018, Ghosh et. al., 2018, Hickey et. al., 2017). CRISPR/Cas9 and CRISPR/Cpf1 (Chen et. al., 2019) are two examples of genome editing technologies that can support effective crop research. The enhancement of desirable traits using genome editing and standard and unconventional plant breeding methods (Voss-Fels et. al., 2019, Watson, et. al., 2018).

The CRISPR/Cas system is a powerful tool for generating rapid genetic variability (Shen et. al., 2017). It possesses multiplexing capabilities, allowing for modifications of multiple targets and the simultaneous stacking of multiple traits within a single generation. Furthermore, the CRISPR/Cas system can induce diversity by targeting and interfering with regulatory elements such as promoters and enhancers (Soyk et. al., 2017). By inhibiting these regulatory factors, CRISPR/Cas has demonstrated improvements in inflorescence architecture and flower production (Liu et. al., 2021).

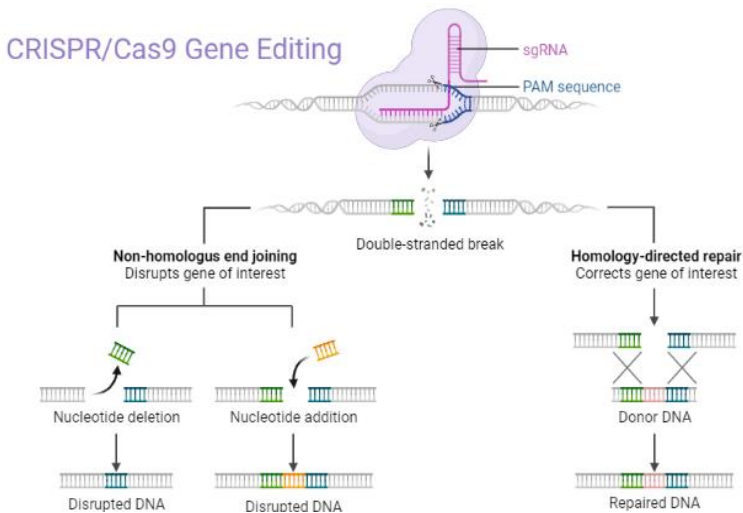


Figure 3. Applications of CRISPR-Cas systems in genome editing

In the context of polyploid crops like potatoes, which possess multiple gene copies, it can be challenging to alter or delete a single target gene through traditional breeding methods without compromising potato quality. In such cases, genome editing platforms like CRISPR-Cas9, particularly suited for polyploid plant genomes, offer a more efficient solution. They enable the addition, deletion, or substitution of the gene of interest. CRISPR-Cas9-mediated editing relies on single guide RNA (sgRNA) to guide the Cas9 enzyme to the specific DNA sequence of interest (Andersson et. al., 2018; Liang et. al., 2017; Svitashhev et. al., 2016).

However, in vitro tissue culture manipulation is necessary for genome-edited plants and the edited plants might still be governed by laws governing transgenic plants. Compared to conventional methods, combining genome editing with speed breeding (SB) can speed up the production of desired varieties. These improvements in tissue culture methods allow us to avoid the legal ramifications of using products that have been genetically modified. For instance, targeted allelic modifications have been successfully achieved through exogenous application of genome-edited constructs using the Cas9 protein. In order to create plants that are resistant to viruses, RNA interference constructs have been delivered using clay nanosheets. Additionally, targeted editing of maize, wheat and potato has been accomplished using CRISPR-Cas9 ribonucleoprotein complexes. Optimizing germination of seedlings and mature seeds is necessary because protoplasts or immature embryos make ideal target tissues (Hamada et. al., 2018). Targeting shoot apical meristems of mature seeds or enabling tissue-

specific editing through biolistic delivery are other methods for delivering such constructs. Other methods include viral vectors (such as the geminivirus group) and particle bombardment in planta (Hamada et. al., 2018). These delivery techniques give the flexibility to create desired variants and speed up the selection process by allowing editing in a variety of tissues, including pollen and inflorescence.

In summary, the combination of Marker Assisted Selection (MAS) with speed breeding (SB), genomic selection (GS) and the CRISPR/Cas system can strengthen accurate selection and contribute to increased genetic gain per year. Additionally, advancements in tissue culture techniques associated with these approaches can help navigate regulatory concerns related to genetically modified products. The integration of these methodologies holds significant potential for expediting the selection of desirable traits and accelerating plant breeding programs.

CONCLUSION AND FUTURE PERSPECTIVES

The potential for speed breeding to revolutionize crop improvement and meet the demands of growing global food needs is immense. The world population is expected to reach 9.7 billion by 2050 and as a result, the demand for food will continue to increase. Speed breeding technology can help meet this demand by accelerating breeding cycles and producing crops with improved traits. By integrating cutting-edge technologies like genomic selection, marker-assisted breeding and the CRISPR/Cas system, speed breeding offers rapid, targeted mutagenesis and the ability to pinpoint specific plant molecular mechanisms for crop improvement. Traditional breeding methods are time-consuming, laborious and untargeted, while speed breeding can produce results quickly and effectively. By extending photoperiods and researching genes in crops that are sensitive to light, speed breeding can be improved and used on a wider variety of crops.

Furthermore, speed breeding can be combined with other strategies like pollen selection, genome editing and high-throughput phenotyping to enhance breeding programs' overall effectiveness. With the development of genome manipulation, next-generation sequencing and genomics, speed breeding can enable extensive plant genotype screening. This means that customized crops with high nutritional value, increased yield and resistance to environmental stresses can be created by using this technology.

Despite the fact that speed breeding requires substantial resources, it is cost-effective in the long run. Therefore, training programs, government support policies, financial aid, the availability of raw materials and infrastructure development should all be put in place to encourage its widespread use. Through

accelerated breeding cycles and improved crop traits, speed breeding technology offers a promising solution for advancing the goals of food security and sustainable agriculture.

In conclusion, speed breeding technology has enormous potential to revolutionize crop improvement and meet the rising global food demand. It is time to invest in this technology and explore its full potential to create a more sustainable future for all.

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