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Review Article

Effects Of Ghost Fishing On Ecosystems and Biodiversity

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Abstract

Ghost fishing is when fishing equipment is lost or abandoned and keeps on catching organisms in an uncontrolled manner in the water body, which creates an environmental impact on aquatic ecosystems and biodiversity. Due to the fact that most fishing equipment is made of durable artificial materials, it tends to stay active in the environment for a considerable amount of time, causing unaccounted mortality to both target and non-target species. Ghost fishing may impose more pressure on endangered species and, in the end, cause restructuring of populations, species extinction, predator-prey relationships, and food web interactions. Ghost fishing affects other organisms in a way different from causing their mortality. Ghost fishing equipment that settles to the bottom of the water or moves along the bottom has the potential to cause physical harm to the benthic ecosystem and may even cause invasion by certain organisms into new areas. Moreover, fishing gear made of synthetic polymers like polyamide, polyethylene, and polypropylene has the potential to break down slowly in the water and cause microplastic pollution by adding plastic to aquatic food webs. Because deaths caused by ghost fishing are not recorded in official catches, it may also contribute to an underestimation of total fishing mortality and fish stock assessment, which could cause faulty fishery management policies. Hence, stopping ghost fishing and the removal of lost or discarded fishing gear are necessary for biodiversity conservation, habitat protection, pollution prevention, and sustainable fisheries management.

Keywords: Ghost fishing, biodiversity, ecosystem, fish stock, fishing gear.

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1. Introduction

Ghost fishing is normally described as the total direct and indirect consequences of abandoned, lost, and other disposed fishing gear (ALDFG) that remain functional after escaping the control of fishers, and thus affect the life of aquatic organisms and habitats. This happens because the current fishing gears are made of robust synthetic polymers, such as PA, PE, and PP, and thus can remain intact in water for long periods and, under certain conditions, continue capturing aquatic organisms. Passive fishing gears, including gillnets, trammel nets, traps, and fyke nets, continue catching targeted and untargeted organisms after being lost, and thus constitute the main sources of ghost fishing.

The effects of ghost fishing gear are not only about the death of the trapped animals, but include other ecological impacts as well, such as habitat destruction, loss of biodiversity, disruption of food chain dynamics, the presence of artificial substances in aquatic habitats, and the spread of non-native species. Recent systematic reviews have found biodiversity loss and habitat destruction as among the major ecological impacts of ghost fishing [1], [2], [3],[4].

Among other effects caused by ghost fishing, there is also the effect of continued and unrestricted fishing of commercial fish and shellfish species. As it has been noted above, the fishing efficiency of passive fishing gear that has been lost and is no longer controlled by fishers can last for some time, which means that the mortality of these organisms caused by the gear is an extra mortality factor that does not show up in official fishing statistics. Organisms caught in such gear may die because of starvation, injuries, diseases, predation, cannibalism, or

unfavorable environmental conditions. Besides, the preservation or decay of dead organisms in the fishing gear may attract more organisms, leading to a continuous cycle of catching and death in some cases [1], [3].

2. Impacts Of Ghost Fishing on Aquatic Ecosystems

However, this decrease in fishing efficiency is often associated with the gradual degradation of the physical integrity of the equipment, its net height, sedimentation, and biofouling. Nonetheless, the decrease in the effectiveness of the gear does not mean that the ecological effects of this fishing equipment will be diminished in a short time. The kind of fishing equipment and its materials, water depth, currents, sea bottom characteristics, as well as the amount of biofouling, may have an important impact on the period and intensity of ghost fishing. In inland water bodies, the lack of sufficient surface area, changes in water levels, high levels of exploitation of the coast, and complicated bottom structures may cause the accumulation of lost fishing gear in certain regions. In addition, small-scale fishing without records, the lack of standardized reporting on losses of fishing gear, and the lack of professional retrieval capabilities prevent the assessment of the real amount of lost fishing gear [5].

Fishing gear may be lost because of various environmental, operational, and socioeconomic factors. Major causes include severe weather conditions, currents, waves, and fluctuations in water levels; entanglement with rocky or obstructed bottom structures; interactions and conflicts with commercial vessels or other fishing activities; failure of buoys or connecting components; inadequate maintenance; operator error; deliberate disposal to eliminate evidence of illegal fishing activities; the absence of appropriate waste-reception facilities at ports; and the costs associated with the disposal of fishing nets that have reached the end of their operational life [6], [7].

Ayaz et al. [8] investigated the ghost-fishing characteristics of monofilament and multifilament gillnets in İzmir Bay and reported that monofilament nets continued fishing for 106 days, whereas multifilament nets remained active for 112 days. The study demonstrated that weekly catch rates decreased exponentially over time; nevertheless, lost nets continued to capture aquatic organisms for several months. Moreover, the overall fish catch rates of monofilament nets were reported to be significantly higher than those of multifilament nets.

A pilot study conducted in Lake Ohrid demonstrated that old and lost fishing nets can accumulate on the lake bottom and can be successfully recovered through targeted retrieval operations [9]. Systematic investigations of lost angling gear have also highlighted the environmental pollution associated with recreational fishing, particularly through the accumulation of fishing lines, hooks, and metal sinkers in aquatic environments [10].

Dartay [11] estimated that approximately 7.2 km of gillnets, 4.4 km of trammel nets, and 500 traps/fyke nets are lost annually in Keban Dam Lake and demonstrated that these passive fishing gears, once lost from fishers' control, may constitute a significant ghost-fishing threat to freshwater species. One of the most important management implications of this uncontrolled mortality is the potential underestimation of actual total fishing mortality. Since individuals that die in ghost fishing gear are not included in commercial landing statistics, such mortality may represent an additional source of uncertainty in stock assessments, particularly in fisheries where fishing gear losses are substantial. Therefore, monitoring mortality associated with abandoned, lost, or otherwise discarded fishing gear (ALDFG) is important for sustainable fish-stock management.

The ecological impacts of ghost fishing gear are not restricted to target species. When fishing gear is operated under the control of fishers, factors such as target species, fishing period, fishing grounds, and gear soak time can be managed to a certain extent. Once fishing gear is lost, however, these control mechanisms are eliminated. Consequently, ghost fishing gear may capture not only fish, crustaceans, cephalopods, and other invertebrates, but also non-target organisms such as seabirds, marine mammals, and sea turtles.

One of the most significant conservation concerns associated with ghost fishing gear is its non-selective interaction with threatened and protected species. Global assessments conducted by FAO and UNEP have identified sea turtles, seabirds, and marine mammals among the major vulnerable groups affected by ALDFG. Synthetic fishing nets that retain their physical integrity for prolonged periods may act as a persistent source of low-level mortality, potentially resulting in substantial consequences for small or vulnerable populations [3].

In the case of the research carried out by Ayaz et al. [8] in İzmir Bay, 29 species of organisms were found in the ghost fishing nets, namely 22 species of fish, five species of crustaceans, one species of cephalopod, and one species of gastropod. The uncontrolled catch of fish in the ghost fishing nets is not limited to the deaths of organisms caught there. The injuries that those who manage to escape suffer can affect their swimming and feeding activities, cause infections, and make them more vulnerable to predators. Hence, while evaluating the environmental effects of ghost fishing, not only the number of organisms died in fishing nets but also their possible sublethal impacts should be considered. Such sublethal impacts include reduced growth, alteration of swimming and feeding behavior, poor reproductive success, oxidative stress, changes in enzyme activities, immune suppression, and tissue injury.

It was noted in the same study that 17 animals of an endangered species, *Pinna nobilis*, had died. This proves that ghost fishing gear is not a threat only to mobile species but could also cause harm to sensitive and sessile species through currents and movements of the fishing gear. Thus, the impact of ghost fishing gear in protected areas, spawning sites, nurseries, seagrasses, reefs, and habitats of highly threatened species is much higher ecologically than an equal quantity of lost fishing gear in areas that are not as sensitive. In other words, the evaluation of ghost fishing gear should not be made solely on the amount of lost fishing gear but also on the sensitivity of the habitat and species interacting with the gear.

Floating nets, buoys, and ropes create artificial substrates upon which bacteria, microalgae, invertebrates, and other organisms can attach and grow. Drifting in currents, the items may transfer the attached organisms out of their natural dispersion ranges and aid biological rafting processes and possibly introduce non-indigenous and invasive organisms. The extent of this risk depends on how long the item remains in the water body, its buoyancy, transport mechanisms, and the vulnerability of the receiving habitat to biological invasions [12].

The role of fishing gear materials in global plastics pollution differs between environmental compartments and geographic regions. The significant share of nets, ropes, and other fishing gear waste detected in offshore oceanic garbage patches indicates that fishing materials can survive in the marine environment and travel long distances [13].

Lost fishing equipment is capable of physically harming benthic habitats as it will move on the bottom of the sea or lakebed, depending on the waves and water currents, as well as entangling in benthic structures. The consequences of such physical interactions might be the abrasion of benthic life forms, their detachment, entanglement in nets, changes in the substrate composition, and movement of the benthic structures to other places. Coral species, hydrocorals, and other benthic life forms that have structures that grow over the substrate are especially susceptible to entanglement with fishing equipment. Contact between ghost gear and coral structures leads to injuries, destruction, death, and modification of benthic structures that offer protection and food to organisms living there. Research has shown that lost fishing gear and lines increase mortality of *Millepora alcicornis* colonies and decrease feeding of herbivorous fish in these colonies. Therefore, the impact of physical harm to benthic habitats is not only limited to those organisms that will directly interact with the gear. Any form of harm to the habitat-forming organisms can also have indirect impacts on other species that rely on such habitats for protection, nutrition, or reproduction purposes. Hence, the ecological impact of ghost fishing gear could go beyond the individual organism level to encompass habitat-level impacts as well [1], [3].

Artificial fishing nets, ropes, and fishing lines may be subject to deterioration and gradually disintegrate into smaller pieces due to ultraviolet (UV) radiation, changes in temperature, wave action, mechanical friction, and biodegradation. Consequently, through this process of degradation, ALDFG can move from entangling marine wildlife through macroplastics to being a possible producer of microplastics. Fragmentation is determined by certain variables, such as type of polymer, chemical additives, time spent in the environment, and hydrodynamic energy. Released into the environment, these pieces can travel through the entire water column, settling in sediments or being ingested by plankton, benthic organisms, and fish, thus entering the aquatic food web [14].

3. Impacts on Biodiversity and Food Webs

The ongoing mortality of both target and non-target species through ghost fishing might have effects on species abundance, composition, and biodiversity. More specifically, the elimination of certain species that serve important roles in nature, including commercially exploited species, predators, herbivores, and habitat builders, might cause changes in species interactions and influence aquatic ecosystem functioning [2], [3].

In addition, fish and invertebrate species captured and killed due to ghost fishing will attract other species of organisms that will come to consume these dead fish and other invertebrates. Subsequent entrapment and capture of these scavenger species by the same ghost fishing gear will start a new cycle of secondary capture events after the first one. Therefore, even after fishing activities stop, the ghost fishing gear will continue to act as a source of artificial mortality for quite a while.

Any destruction of benthic habitats might further increase the impact of ghost fishing indirectly on biodiversity. The destruction of coral reefs, seagrass beds, and other structurally complex habitats may result in a decrease in habitats for juveniles, invertebrates, and feeding grounds for many fish species. Indeed, systematic and bibliometric analysis conducted recently has revealed that biodiversity loss and habitat destruction are the main ecological impacts of ghost fishing [2], [4].

Furthermore, the long-term and ecosystem-scale impacts of ghost fishing gear on food webs may ultimately be reflected in the degradation of ecosystem services, including food provision, habitat support, recreational opportunities, aesthetic values, and safe navigation [4].

9. Conclusion

In summary, the negative effects of ghost fishing on aquatic ecosystems need to be considered an environmental issue that goes far beyond the continued catching of fish by lost fishing equipment. Unreported deaths of target fish species, the catching and death of non-target and protected species, habitat damage, indirect impact on biodiversity and trophic interactions, plastic and microplastic pollution, and transportation of invasive species are the different facets of the same environmental issue. Consequently, ghost fishing management needs to be approached using an ecosystem-based perspective, which includes the prevention of fishing equipment loss, quick detection and retrieval of the lost fishing equipment, and focusing on the retrieval of lost equipment in sensitive ecosystems, among other measures.

Some of the key knowledge gaps include the poor representation of inland water and artisanal fisheries in the current body of literature, lack of standard approaches to measure rates of fishing gear losses, uncertainty about the connection between ghost fishing period and overall mortality, insufficient studies on the impact of ghost fishing on sub-lethal and population levels, inability to identify microplastics as a product of fishing gear, and a lack of evaluation of the efficiency of management and retrieval actions in the long run.

Future research should categorize the reasons for lost fishing gears based on the types of gear and regions, include suitable control areas in their research design, document the temporal changes in the physical status and fishing efficiency of lost gears, and at the same time measure the mortality rates of the target, non-target, and protected species. Moreover, stock assessment models should take into account the number of lost gears, the period in which the gears are still fishing, and uncertainties related to their catching efficiency. The application of such models will contribute to the accurate estimation of the total fishing mortality rate and help develop mitigation strategies to address ghost fishing.

Author Contributions

Conceptualization, Writing, Submission.

Conflict of Interest

The author(s) declare no conflicts of interest.

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Research Article

Green Cobalt Nanoparticle Synthesis, Characterisation, Magnetic Properties, and Phytostimulatory Impacts of *Moringa oleifera* Leaf Extract

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Abstract

The distinctive structural, chemical, magnetic, and biological characteristics of cobalt nanoparticles (CoNPs) have garnered significant interest. In this work, *Moringa oleifera* leaf extract and cobalt nitrate solution were used in an eco-friendly green synthesis method to create cobalt nanoparticles. The crystalline structure, functional groups, and magnetic behavior of the synthesized CoNPs were examined using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and vibrating sample magnetometry (VSM). Characteristic diffraction peaks were identified by XRD analysis with 2θ values of 36.2° , 43.1° , 50.4° , and 73.9° , corresponding to the (311), (400), (422), and (533) planes. The diffraction pattern matched JCPDS card No. 22-1086 and was compatible with the stated cubic spinel structure. The Scherrer equation yielded an average crystallite size of 18.37 nm. Along with Co–O-related vibrations, FT-IR analysis showed absorption bands at 3376, 1656, 591.99, and 495 cm^{-1} , suggesting the role of hydroxyl-containing and other functional groups in the production and stabilization of the nanoparticles. The produced CoNPs' magnetic nature was validated by VSM analysis. Canola plants were used to assess the synthesized nanoparticles' biological and agronomic applications. At lower concentrations, CoNP treatment increased shoot fresh weight, shoot dry weight, and shoot length; the greatest growth response was shown at 50 ppm. Plant growth decreased at higher doses, suggesting that CoNPs may have a concentration-dependent harmful impact. These results show that magnetic cobalt-based nanoparticles with potential uses in nanotechnology, agriculture, and biomedicine may be produced easily and sustainably by *Moringa oleifera*-mediated synthesis. These results show that magnetic cobalt-based nanoparticles with potential uses in nanotechnology, agriculture, and biomedicine may be produced easily and sustainably by *Moringa oleifera*-mediated synthesis.

Keywords: XRD, FT-IR, VSM, cobalt nanoparticles, green synthesis, *Moringa oleifera*, canola, plant growth, and magnetic nanoparticles.

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1. Introduction

The production, characterization, and use of materials with dimensions typically falling within the nanoscale range are all part of the growing multidisciplinary topic of nanotechnology. Because of their small particle size, huge surface-to-volume ratio, and size-dependent structural and electrical features, nanomaterials can have physicochemical properties that are significantly different from those of their bulk counterparts. Cobalt-based nanoparticles are particularly significant among metallic and metal-oxide nanomaterials due to their optical, biological, magnetic, and catalytic characteristics [1], [2]. For use in magnetic materials, catalysis, sensing,

biomedical technology, and environmental remediation, cobalt nanoparticles have garnered growing scientific attention. Applications requiring magnetic separation, targeted delivery systems, imaging-related technologies, and other magnetically controlled processes find them especially appealing due to their magnetic characteristics. The size, crystal structure, shape, surface chemistry, and magnetic properties of cobalt nanoparticles all have a significant impact on their performance [3],[4].

High temperatures, high pressures, hazardous reducing agents, organic solvents, and other ecologically undesirable substances can all be used in conventional methods for the production of nanoparticles. These methods may raise the expense and environmental impact of producing nanoparticles, as well as produce harmful byproducts. As a result, green synthesis has drawn a lot of interest as a different method for creating nanoparticles [1], [4], [5]. Because plant extracts include naturally occurring biomolecules that can take part in the reduction, nucleation, growth, and stabilisation of nanoparticles, plant-mediated synthesis is one of the most appealing green synthesis techniques. Plant extracts contain polyphenols, flavonoids, proteins, carbohydrates, organic acids, and other phytochemicals that can interact with metal ions and aid in the creation of nanoparticles. Plant-mediated synthesis can offer a rather easy and affordable method for producing nanoparticles in addition to being ecologically benign [3], [6]. Numerous physiologically active phytochemicals may be found in the well-researched medicinal and nutritious plant *Moringa oleifera*. Numerous substances found in its leaves may contribute to the stabilisation and reduction of metal nanoparticles. *Moringa oleifera* leaf extract is therefore a biological substance that shows promise for the environmentally friendly production of nanomaterials [4], [7]. The current work used a green synthesis method to create cobalt nanoparticles utilising leaf extract from *Moringa oleifera*. FT-IR spectroscopy was used to detect significant functional groups linked to the synthesised nanoparticles, VSM was used to examine their magnetic behaviour, and XRD was used to examine their crystal structure and estimate their crystallite size. Additionally, the impacts of the synthesised CoNPs on certain canola plant development indices, such as shoot fresh weight, shoot dry weight, and shoot length, were assessed [5], [7], [8].

Therefore, the study's goals were to create cobalt nanoparticles utilising a green synthesis method and leaf extract from *Moringa oleifera*. Use XRD to examine the crystallite size and structural characteristics of the produced CoNPs [9]. Use FT-IR spectroscopy to identify significant functional groups connected to the produced nanoparticles. Using VSM, ascertain the magnetic characteristics of the produced CoNPs. Examine how various CoNP concentrations affect particular canola plant development characteristics. Analyze the produced CoNPs' potential for use in agriculture and other technical fields [7], [8], [10].

2. Materials and Methods

2.1. Green Cobalt Nanoparticle Synthesis and Characterizations

A green synthesis method was used to mix the plant extract with a 1 M cobalt nitrate solution. The leaf extract's phytochemical components reacted with the cobalt precursor to promote the creation of nanoparticles. After that, the material was treated to produce powdered cobalt nanoparticles for further analysis. Three main methods were used to characterize the synthesized CoNPs: Fourier transform infrared spectroscopy, vibrating sample magnetometry, and X-ray diffraction. Confirmation of magnetism was carried out using VSM (Lakeshore, 7407 USA). For XRD, a Bruker D8 Advance X-ray diffractometer ($\text{CuK}\alpha$: $\lambda = 1.5407 \text{ \AA}$; $1.5^\circ/\text{min}$ scanning rate: $40 \text{ kV}/35 \text{ mA}$) was used. MRI was carried out using a Siemens 0.35T MRI open MRI (Hi Tech, USA).

2.2. X-Ray Diffraction Analysis

The crystalline structure of the synthesized material was studied by X-Ray diffraction (XRD) analysis. Bruker-D8 Advance Laboratory Diffractometer (with $\text{CuK}\alpha 1$ radiation, $\lambda = 1.54 \text{ \AA}$). XRD analysis along with the Scherrer equation ($D_{hkl} = k \lambda / \beta \cos \theta$) was employed to study phase identification, crystallinity, and average particle size [11].

2.3. Vibrating Sample Magnetometer Analysis

A vibrating sample magnetometer (VSM) was used to verify the synthetic material's magnetic properties. According to the previous protocol, the Saturation magnetization, Retentivity, and Coercivity of the hysteresis loop were studied with a VSM (Lakeshore 7407) [12], [13].

3. Results

3.1. Size and Morphology Analysis

The size and structural composition of a single unit cell are examined using the X-ray diffraction method. For X-ray diffraction, the generated Cobalt nanoparticles (CoNPs) were used in powder form.

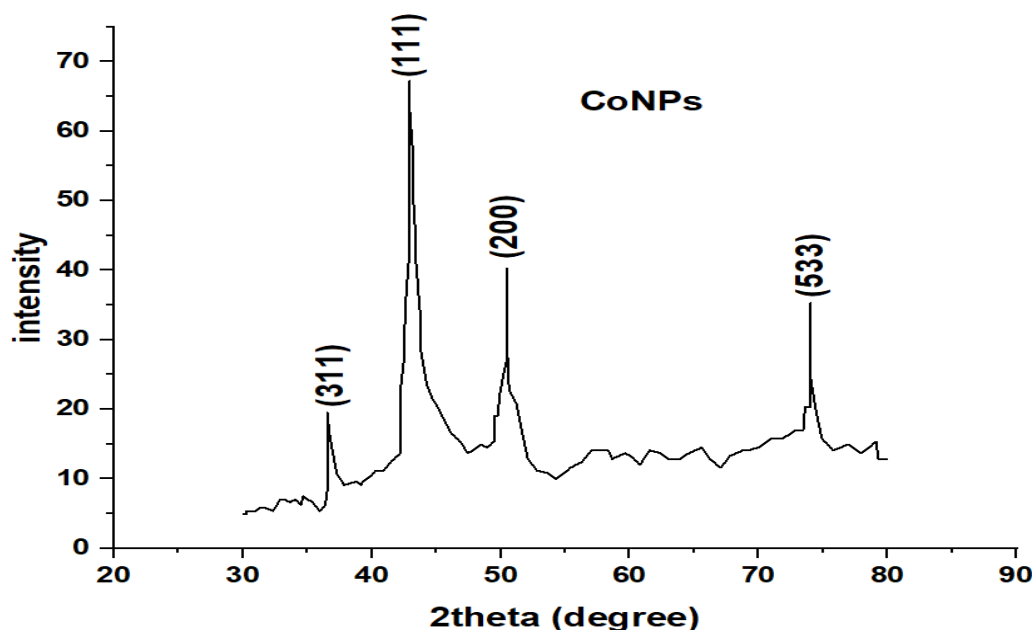


Figure 1. XRD pattern of the produced CoNPs.

The XRD pattern of the produced CoNPs is shown in Figure 1, with diffraction peaks at angles of 36.70, 43.10, 50.40, and 73.90. The hkl values corresponding to these peaks are (311), (400), (422), and (533). The XRD card number 22-1086 matches all of the produced sample's peaks and their locations on the 2theta scale, which are compatible with the distinctive cubic spinel peaks [1], [13], [14]. It was discovered that the cobalt crystallite size was 18.3 ± 7 nm.

3.2. Fourier Transform Infrared Spectroscopy Analysis

The relationship between different species and modifications in the mixture's chemical structure is investigated using Fourier transform infrared spectroscopy. Figure 2 displays the infrared spectra of CoNPs obtained using the green method, which were recorded in the 400–4000 cm^{-1} range. CoNPs made using the green synthesis approach exhibit absorption peaks at 3376, 1656, 591.99, and 495 cm^{-1} , respectively, which represent the stretching vibration of Co-O, the bending mode of the water molecule, and O-H stretching [15], [16].

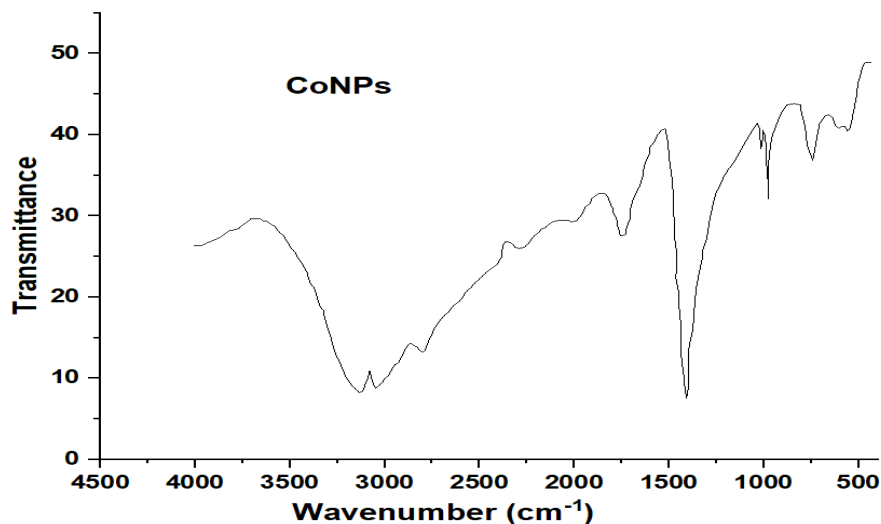


Figure 1. FTIR analysis of Cobalt nanoparticles using *Moringa oliefera* leaves extract.

3.3. Vibrating Sample Magnetometer

The magnetic properties of the produced Cobalt nanoparticles made from the extract of *Moringa oliefera* leaves were investigated using VSM analysis. Figure 3 displays the magnetization vs. magnetic field curve [16], [17]. The magnetic property of the synthesized cobalt nanoparticles is supported by their maximum saturation magnetization of 24.083 emu/s. The ferromagnetic nature of these synthetic cobalt nanoparticles is demonstrated by the VSM analysis graph. Cobalt nanoparticles have a coercivity of 495 Oe and a retentivity of 8.606 emu/g. Soft magnetic materials are anticipated to be used in a variety of biological applications, including gene transfer, immunological testing, cell separation, magnetic resonance imaging (MRI), and drug administration.

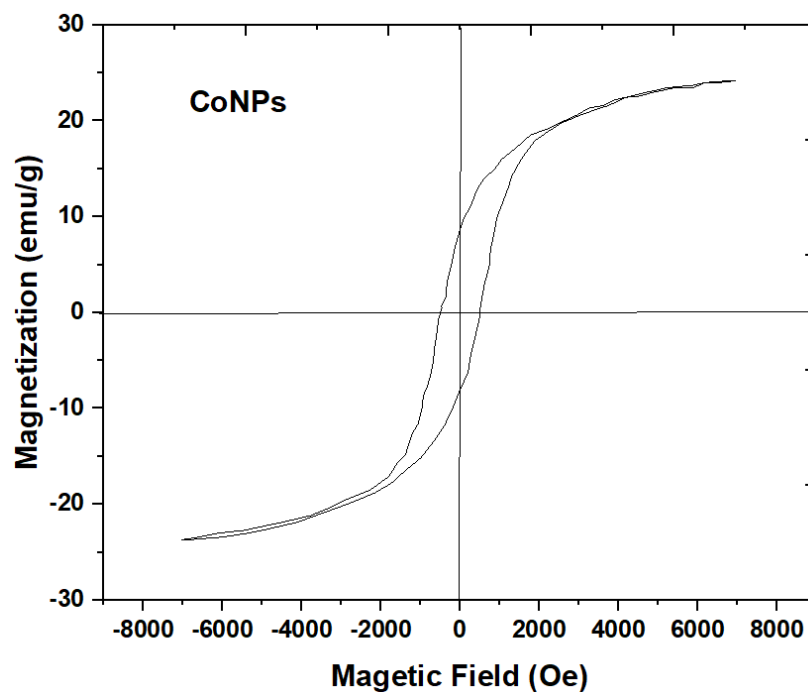


Figure 3. VSM analysis of Cobalt nanoparticles using *Moringa oliefera* leaves extract.

3.4. Effect of Cobalt Nanoparticles on Canola Plant Growth

The impact of the synthesized CoNPs on certain canola plant development traits was further examined. Shoot fresh weight, shoot dry weight, and shoot length were the factors that were assessed. The findings show that the reaction is concentration-dependent. Plant growth was typically enhanced by lower concentrations of CoNPs, but growth characteristics decreased when the concentration was raised above the optimal threshold. This behaviour suggests that, whereas cobalt nanoparticles may be advantageous at suitable doses, they may become harmful at higher doses. When considering nanoparticles for agricultural uses, such concentration-dependent reactions are crucial. Because it reflects the mass of recently harvested plant tissue, shoot fresh weight is a crucial metric for evaluating plant development and biomass buildup.

Plants treated with 50 ppm CoNPs (T1) had the maximum fresh weight of canola shoots, measuring about 2.75 g plant⁻¹. The fresh weight of plants treated with 100 ppm CoNPs (T2) was around 2.60 g plant⁻¹. By contrast, the fresh weight of about 2.30 g plant⁻¹ was the lowest for the untreated control (T0). These findings show that, in comparison to the untreated plants, CoNP treatment at comparatively low concentrations increased the generation of fresh biomass. The increased fresh weight could suggest that plant physiological processes can be positively impacted by a suitable concentration of CoNPs. The decrease at greater concentrations, however, indicates that there may be an optimal concentration for the positive impact, after which plants may experience stress from nanoparticle exposure.

9. Discussion

The results of this study show that *Moringa oleifera* leaf extract may be used to successfully synthesize cobalt-based nanoparticles utilizing a green synthesis method. Using the Scherrer equation, XRD examination yielded an average crystallite size of 18.37 nm, confirming the synthesized material's crystalline nature [2], [16], [18]. The existence of significant functional groups connected to the synthesized substance was shown by the FT-IR data. Oxygen-containing vibrations and the O–H-related band show how phytochemical components from *Moringa oleifera* leaves contribute to the synthesis and stabilization of nanoparticles. Co–O vibrations were linked to the low-wavenumber bands that were seen.

The magnetic nature of the produced nanoparticles was further shown by the VSM data. Retentivity of 8.606 emu/g, coercivity of 495 Oe, and saturation magnetization of 24.083 emu/g were recorded [7], [15], [19]. These characteristics lend credence to the synthetic material's possible usage in magnetic applications. The plant-growth experiment showed that the nanoparticles can also have concentration-dependent effects on biological systems. In comparison to the untreated control, canola shoots treated with reduced CoNP concentrations had better fresh weight, dry weight, and shoot length. At 50 ppm, the greatest reaction was often seen. However, plant growth was significantly reduced at higher doses, especially 500 ppm.

An interesting finding for the possible application of nanoparticles in agriculture is this concentration-dependent reaction. At the right doses, nanomaterials may have positive benefits, but too much exposure might cause physiological stress and stunted growth. Therefore, prior to actual agricultural use, nanoparticle concentration optimisation is crucial. Cobalt nanoparticles produced by *Moringa oleifera* show promise as a multifunctional nanomaterial due to their green production, nanoscale crystallite size, magnetic behaviour, and biological activity. However, further research is needed to determine their exact magnetic structure, shape, elemental composition, surface chemistry, and long-term biological impacts.

Author Contributions

All authors contributed equally to conceptualization, methodology, writing, and editing.

Conflict of Interest

The authors declare no conflicts of interest.

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Review Article

Aquamind-AI: An Intelligent IoT and Machine Learning Framework For Closed-Loop Smart Aquaponics

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Abstract

Global freshwater depletion, accelerating climate volatility, and arable soil salinization pose existential constraints on conventional agro-aquacultural production. Aquaponic – a circular polyculture biotechnology coupling Recirculating Aquaculture Systems (RAS) with soil-less hydroponics—presents an environmentally sustainable paradigm by recovering toxic metabolic ichthyological excretions as primary vegetative biofertilizer. However, the operational equilibrium of aquaponic systems is governed by tightly coupled, non-linear biological dynamics across finfish, autotrophic nitrifiers (*Nitrosomonas* and *Nitrobacter*), and plant root interfaces. Transient deviations in physicochemical parameters (such as water temperature, dissolved oxygen, and hydrostatic levels) induce rapid ecosystem collapse, hypoxic asphyxiation, or systemic nitrite poisoning. To resolve these multidimensional vulnerabilities, this paper delivers a comprehensive, scientifically grounded review of smart aquacultural methodologies and introduces AquaMind-AI, an open-architecture, edge-to-cloud cyber-physical framework engineered for autonomous aquaponic governance. AquaMind-AI integrates a low-cost, fault-tolerant sensory edge layer (incorporating digital single-wire DS18B20 thermal probes, non-contact HC-SR04 ultrasonic echo-ranging hydro-level sensors, and DHT11 ambient microclimate transmitters managed by ATmega328P edge microcontrollers) with galvanically isolated, optocoupled commutation stages driving 6V DC hydraulic recirculation pumps and 220V AC high-output aeration compressors. The computational middleware leverages an asynchronous FastAPI microservice backend coupled with an ensemble Random Forest regressor with harmonic diurnal feature engineering for multi-horizon thermal forecasting ($R^2 = 0.942$ for 1h, $R^2 = 0.854$ for 6h), an unsupervised multivariate Isolation Forest algorithm for zero-day sensor outlier isolation, and a dynamic piecewise Health (Shealth) and Risk (Rsystem) scoring engine. We demonstrate that AquaMind-AI achieves proactive anomaly mitigation, eliminates catastrophic failure modes, and establishes a validated, scalable blueprint for climate-adaptive food security in arid and semi-arid geographies such as the Caspian Basin and Kura-Aras lowlands.

Keywords: adaptive closed-loop control, anomaly detection, Caspian basin, Internet of Things (IoT), isolation forest, precision aquaculture, random forest, sustainable agritech.

1. Introduction

The 21st-century agricultural sector is confronted with a severe global sustainability crisis. Global population projections indicate a trajectory toward 9.7 billion inhabitants by 2050, requiring an estimated 60–70% increase in global food production under conditions of unprecedented climatic volatility, land degradation, and acute freshwater scarcity [1], [2]. Conventional flow-through aquaculture consumes extensive volumes of pristine

freshwater and discharges nutrient-dense wastewater rich in ammonia, phosphorus, and organic suspended solids, directly driving freshwater eutrophication and aquatic habitat destruction. Concurrently, traditional intensive soil agriculture accounts for over 70% of global anthropogenic freshwater withdrawals and relies heavily on energy-intensive synthetic nitrogenous fertilizers that cause severe groundwater contamination and greenhouse gas emissions [3].

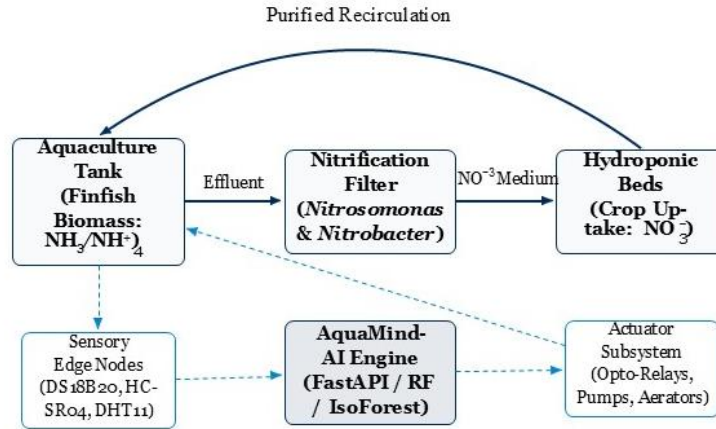


Figure 1. Integrated biological recirculation loop and superimposed AquaMind-AI cyber-physical monitoring and control topology.

In response to this resource nexus, aquaponics has emerged as an exceptionally promising closed-loop circular bioeconomy technology. As shown in Figure 1, aquaponics integrates Recirculating Aquaculture Systems (RAS) with soilless hydroponic plant cultivation within a single unified thermodynamic and biological loop. Total ammonia nitrogen (TAN), excreted via finfish gills and fecal mineralization, exists in an aqueous temperature- and pH-dependent thermodynamic equilibrium between un-ionized ammonia (NH_3 , highly toxic to aquatic life at concentrations $> 0.05 \text{ mg/L}$) and ionized ammonium (NH_4^+ , non-toxic):



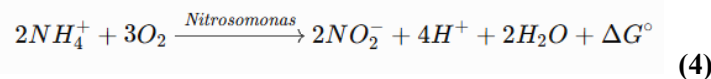
The fraction of toxic un-ionized ammonia f_{NH_3} is mathematically governed by the Henderson-Hasselbalch relation:

$$f_{\text{NH}_3} = \frac{1}{10^{(pK_a - \text{pH})} + 1} \quad (2)$$

where

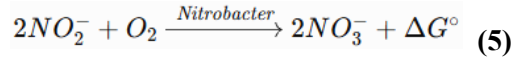
$$pK_a = \frac{2729.92}{T_{\text{water}} + 273.15} + 0.09018 \quad (3)$$

Within the aerobic biological filter, chemoautotrophic ammonia-oxidizing bacteria (AOB, primarily *Nitrosomonas*) oxidatively transform ammonium into nitrite (NO_2^-), an intermediate metabolite that induces methemoglobinemia (brown blood disease) in finfish by irreversibly oxidizing functional ferrous hemoglobin (Fe^{2+}) into ferric hemoglobin (Fe^{3+}):



where $\Delta G^\circ \in [-652, -548]$ kJ/mol.

Subsequently, nitrite-oxidizing bacteria (NOB, primarily *Nitrobacter* and *Nitrospira*) rapidly oxidize nitrite into non-toxic nitrate (NO_3^-):



where $\Delta G^\circ \in [-155, -143]$ kJ/mol. The resulting nitrate-rich aqueous stream is directed to the hydroponic grow beds, where crop root networks absorb dissolved inorganic nitrogen (NO_3^-) alongside phosphorus, potassium, and micronutrients, thereby bio-filtering the water before it is recirculated back to the aquatic rearing tanks. This closed-loop bio-assimilation conserves upwards of 90–95% of water compared to open-field farming [4].

2. The Biological Fragility Trilemma and Research Motivation

Despite its significant theoretical advantages, aquaponics is characterized by high biological vulnerability. The system represents a multi-trophic polyculture comprising three disparate biological kingdoms—aquatic vertebrates (finfish), microbiological nitrifiers, and vascular plants—each possessing distinct, conflicting physicochemical optima:

1. Finfish Optima (e.g., *Oreochromis niloticus*, commonly known as Nile tilapia): Prefer water temperatures of 26–29°C, dissolved oxygen (DO) levels > 5.0 mg/L, and neutral to slightly alkaline pH (7.0–8.0).
2. Autotrophic Nitrifiers: Require water temperatures of 24–28°C, DO > 3.0 mg/L, and alkaline pH (7.5–8.5) for optimal enzymatic activity of ammonia monooxygenase (AMO).
3. Hydroponic Crops (e.g., *Lactuca sativa*, herbs): Exhibit optimal nutrient uptake and root respiration at lower root-zone temperatures (18–22°C) and slightly acidic pH (5.8–6.5), as essential micronutrients (iron, manganese, zinc, boron) precipitate out of solution at pH > 7.2.

Because commercial operations must balance these conflicting demands within a single recirculating fluid loop (typically targeting pH 6.8–7.2 and water temperature 22–26°C), the system operates near critical physiological tolerance limits. A localized failure (such as undetected pump cavitation, thermal spike, or aeration failure) can lead to rapid hypoxic asphyxiation or toxic nitrite accumulation in under three hours [5].

Manual water sampling and discrete testing cannot detect rapid transient environmental shocks. Existing commercial SCADA systems remain cost-prohibitive for small and medium-scale growers, while basic IoT hobbyist setups lack predictive analytics and multivariate fault isolation. To bridge this critical technological gap, this paper presents the complete scientific framework and empirical validation of AquaMind-AI, an open-architecture, intelligent cyber-physical system designed for real-time monitoring, multi-horizon machine learning thermal forecasting, multivariate anomaly isolation, and closed-loop actuation in climate-vulnerable aquaponic systems.

3. State of the Art in Smart Aquaponics: Sensorics, Microcontrollers, and Actuation

The evolution of automated recirculating agro-aquatic systems spans four distinct technological generations, as summarized in Table 1.

Table 1. Evolutionary paradigms of recirculating aquaculture and aquaponic monitoring.

Generation	Era	Sensing Method	Control Topology	Key Vulnerabilities & Constraints
Aquaponics 1.0	1980–2000	Manual titrations, chemical kits	Manual valves	High labor overhead; zero nocturnal observability; delayed catastrophic response
Aquaponics 2.0	2000–2012	Standalone digital probes	Dedicated logic controllers	Isolated data silos; no remote access; localized analog drift
Aquaponics 3.0	2012–2020	Cloud IoT, basic telemetry	Hard-coded threshold relays	High false-alarm rates; network latency sensitivity; lack of predictive insight
Aquaponics 4.0	2020–Present	Edge-AI, multi-sensor IoT	Predictive, adaptive closed loop	AquaMind-AI paradigm: self-tuning, multivariate anomaly isolation, horizon forecasting

3.1. Physical Sensory Metrology and Circuit Design

Sensory components in aquaponic systems face severe physical constraints, including continuous aqueous submersion, galvanic corrosion, biofilm accumulation, and electrical noise from switching inductive loads (pumps and aerators). AquaMind-AI incorporates an edge sensory array engineered for maximum reliability and electrical isolation (Figure 2).

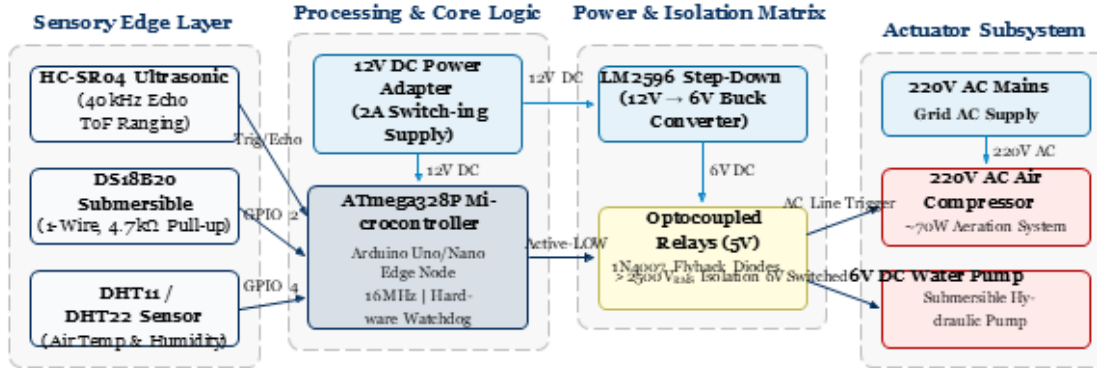


Figure 2. Electrical wiring schematic, sensory instrumentation, and power distribution topology of the AquaMind-AI edge node.

- **Submersible Water Thermometry (DS18B20):** Encapsulated in a 316L waterproof stainless-steel housing (6×50 mm), the DS18B20 communicates via the Dallas single-wire (1-Wire) protocol. An internal 12-bit delta-sigma ADC resolves temperatures to 0.0625°C across a -10°C to $+85^\circ\text{C}$ range with an accuracy of $\pm 0.5^\circ\text{C}$. A $4.7\text{ k}\Omega$ pull-up resistor stabilizes the open-drain bidirectional line against capacitive signal degradation over cable runs up to 10 m.
- **Hydrostatic Ultrasonic Level Ranging (HC-SR04):** Positioned above the sump reservoir, the HC-SR04 emits an 8-cycle burst of 40 kHz ultrasonic pulses and measures round-trip time-of-flight (Δt). Instantaneous water depth h_{water} is calculated by subtracting the measured distance from the total tank height H_{tank} , with air-temperature speed compensation:

$$h_{\text{water}} = H_{\text{tank}} - \frac{(331.3 + 0.606 T_{\text{air}}) \Delta t}{2} \quad (6)$$

This non-contact configuration prevents biofilm accumulation and probe corrosion typical of submerged resistive sensors.

- **Ambient Microclimate Probes (DHT11/DHT22):** Measure ambient air temperature (T_{air}) and relative humidity (RH) within the greenhouse boundary layer. These values enable calculation of the Vapor Pressure Deficit (VPD), a critical indicator of crop transpiration stress and evaporative heat loss:

$$VPD = 0.61078 \exp\left(\frac{17.27 T_{\text{air}}}{T_{\text{air}} + 237.3}\right) \left(1 - \frac{RH}{100}\right) \quad (7)$$

3.2. Microcontroller Firmware and Actuator Commutation

Edge processing is executed on an ATmega328P microcontroller running custom C++ firmware. Polling routines execute on non-blocking hardware timer interrupts ($f_s = 0.1\text{ Hz}$, 10 s intervals), with median filtering across 5 consecutive samples to eliminate acoustic multipath reflections and line transients. An internal Hardware Watchdog Timer (WDT) configured for 8 s provides automatic system recovery in the event of bus lockup.

Actuator drive circuits control two critical electromechanical subsystems:

1. Hydraulic Circulation Subsystem: A 6V DC submersible water pump driven by a dedicated power stage. A 12V DC switching power supply (2A) is stepped down to 6.0V DC via an LM2596 high-efficiency buck converter (or boosted from 5V via an MT3608 step-up converter). Switching is controlled by an optocoupled relay channel or a high-current logic-level N-channel MOSFET (IRF520 / TIP122 transistor driver).
2. High-Output Aeration Subsystem: A 220V AC laboratory-grade diaphragm vacuum/air compressor (~ 70W, equipped with a mechanical manometer and filtration assembly) supplies micro-bubble dissolved oxygen to the biofilter and fish tank. The 220V AC inductive load is isolated from the 5V logic board using an optocoupled electromagnetic relay (isolation voltage > 2500VRMS). Parallel 1N4007 flyback diodes and RC snubber networks suppress inductive voltage spikes ($V = -L \, di/dt$), protecting against logic resets and relay contact degradation.

4. Multi-Tier Cyber-Physical Architecture of AquaMind-AI

AquaMind-AI is structured across a decoupled, three-tier software architecture designed for sub-second diagnostic response and zero telemetry loss (Figure 3).

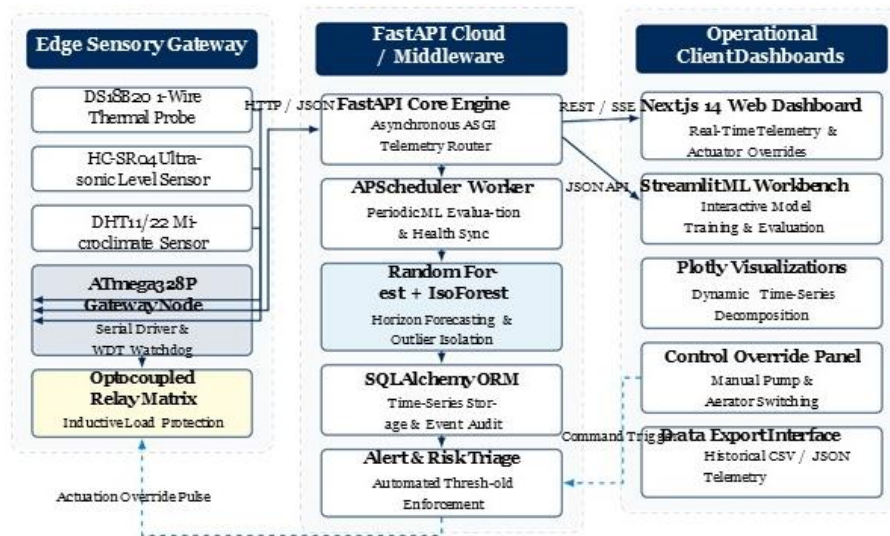


Figure 3. End-to-end data pipeline, microservice topology, and closed-loop feedback pathways in AquaMind-AI.

4.1. Asynchronous Middleware Layer (FastAPI)

The intermediate compute tier is powered by Python 3.11 utilizing FastAPI as an asynchronous web framework on top of the Uvicorn ASGI server. Using asynchronous coroutines ('async/await'), the server concurrently manages incoming edge IoT sensor packets, evaluates real-time alert boundaries, runs machine learning inference models, and streams telemetry to connected client dashboards with low latency (< 15 ms).

Data persistence is abstracted through SQLAlchemy ORM, managing relational data structures for sensor time-series, system alerts, predictive inference snapshots, and actuator state logs. The internal Advanced Python Scheduler (APScheduler) coordinates background maintenance tasks, database integrity verification, and periodic ML model re-training.

4.2. Dual Operational Interfaces

The platform provides two complementary user interfaces tailored for distinct operational requirements:

- Real-Time Operations Dashboard (Next.js 14): A responsive, client-side web application built with React, TypeScript, and Tailwind CSS. It provides live metric gauges, dynamic status indicators, active alert acknowledgment, and manual actuator control overrides.

- Analytical Workbench (Streamlit): A dedicated scientific interface designed for exploratory data analysis, interactive machine learning model training, historical time-series decomposition, and automated CSV/JSON data export.

5. Machine Learning Methodologies and Mathematical Formulations

5.1. Harmonic Diurnal Feature Engineering

Water temperature dynamics in greenhouses follow a distinct 24-hour diurnal cycle driven by solar radiation. To prevent artificial discontinuities associated with standard integer hour representations (e.g., the step from 23:59 to 00:00), raw timestamps are mapped onto orthogonal circular trigonometric coordinates.

$$x_{\sin}(t) = \sin\left(\frac{2\pi h(t)}{24}\right), \quad x_{\cos}(t) = \cos\left(\frac{2\pi h(t)}{24}\right) \quad (8)$$

where $h(t) \in [0, 24)$ is the fractional hour of the day.

To model thermal inertia and dynamic memory in recirculating water volumes, autoregressive lag terms and moving averages are constructed.

$$T_{\text{lag1}}(t) = T_{\text{water}}(t - \Delta t) \quad (9)$$

$$T_{\text{roll3}}(t) = \frac{1}{3} \sum_{k=0}^2 T_{\text{water}}(t - k\Delta t) \quad (10)$$

$$T_{\text{lag1}}(t) = T_{\text{air}}(t - \Delta t) \quad (11)$$

The synthesized feature vector $\mathbf{x}_t \in \mathbb{R}^5$ is defined as:

$$\mathbf{x}(t) = [x_{\sin}(t), x_{\cos}(t), T_{\text{lag1,water}}(t), T_{\text{roll3,water}}(t), T_{\text{lag1,air}}(t)]^T \quad (12)$$

Features are scaled via standard Z-score normalization before model input:

$$z_j = \frac{x_j - \mu_j}{\sigma_j}, \quad \forall j \in \{1, \dots, 5\} \quad (13)$$

5.2. Multi-Horizon Temperature Forecasting (Random Forest Regressor)

Thermal stability is critical in closed-loop aquaponics. To forecast multi-step forward thermal trajectories ($\tau \in \{1h, 3h, 6h\}$), AquaMind-AI employs an ensemble Random Forest Regressor comprising $M = 100$ bootstrap-aggregated decision trees $\{T_m\}^M$; $m = 1, \dots, M$

The ensemble prediction $\hat{y}(t + \tau)$ is formulated as the mean prediction across all independent trees:

$$\hat{T}_{\text{water}}(t + \tau) = \frac{1}{M} \sum_{m=1}^M T_m(\mathbf{x}(t); \Theta_m) \quad (14)$$

where Θ_m denotes the structural split parameters, selected features, and leaf node values for tree m , optimized during training by minimizing the Mean Squared Error (MSE):

$$\mathcal{L}_{\text{MSE}} = \frac{1}{N} \sum_{i=1}^N (T_{\text{water}}^{(i)} - \hat{T}_{\text{water}}^{(i)})^2 \quad (15)$$

5.3. Unsupervised Multivariate Anomaly Detection (Isolation Forest)

Physical failures in aquaculture systems (such as air compressor membrane fatigue, pump cavitation, drain clogging, or biofilm accumulation on probes) often generate multivariate anomalies that do not immediately exceed static univariate threshold limits.

AquaMind-AI implements an unsupervised Isolation Forest model [6]. The algorithm isolates anomalous points by recursively generating random axis-aligned partitions across the

four-dimensional feature space $X \subset \mathbb{R}^4$ (spanning water temperature, water level, air temperature, and air humidity). Anomalous observations require significantly shorter traversal paths from root to leaf in an isolation tree (iTree).

The anomaly score $s(\mathbf{x}, n)$ across a dataset of n instances is formulated as:

$$s(\mathbf{x}, n) = 2^{-\frac{\mathbb{E}[h(\mathbf{x})]}{c(n)}} \quad (16)$$

where $h(\mathbf{x})$ is the path length (number of partition edges traversed), $\mathbb{E}[h(\mathbf{x})]$ is the expected path length across the 100-tree ensemble, and $c(n)$ is the average path length of unsuccessful searches in a Binary Search Tree (BST):

$$c(n) = 2 [\ln(n-1) + \gamma] - \frac{2(n-1)}{n} \quad (17)$$

Here, $\gamma \approx 0.5772156649$ (Euler-Mascheroni constant)

Instances yielding $s \rightarrow 1.0$ (score > 0.60) are flagged as multivariate anomalies (`is_anomaly = True`), triggering an immediate diagnostic alert.

5.4. Dynamic System Health and Risk Metrics

To provide operators with an intuitive, unified operational index, AquaMind-AI calculates a parametric Health Score ($S_{\text{health}} \in [0, 100]$) and a continuous Risk Score ($R_{\text{system}} \in [0, 100]$):

$$S_{\text{health}}(t) = \max \left(0, 100 - \sum_{j \in P} w_j I(v_j(t) \notin [\theta_{\min}, \theta_{\max}]) \right) \quad (18)$$

where $P = \{\text{water_temp}, \text{water_level}, \text{air_humidity}\}$, $W_{\text{water_temp}} = 25$, $W_{\text{water_level}} = 25$, $W_{\text{air_humidity}} = 10$, and $I(\cdot)$ is the binary indicator function.

Complementarily, the continuous Risk Score quantifies distance-to-threshold deviation:

$$R_{\text{system}}(t) = \min \left(100, 30|V| + 4 \sum_{v \in V} \delta(v) \right) \quad (19)$$

where V is the set of active sensor threshold violations, and $\delta(v)$ is the magnitude of parameter deviation beyond the safety boundary.

6. Experimental Evaluation, Verification, and Benchmarking

6.1. Operational Parameter Thresholds

Table 2 delineates the operational thresholds configured within AquaMind-AI for warm-water finfish (e.g., Tilapia) and hydroponic leafy greens (*Lactuca sativa*).

Table 2. Physicochemical operating ranges and critical threshold matrices.

Parameter	Hardware Sensor	Unit	Crit. Min	Norm. Min	Norm. Max	Crit. Max
Water Temperature	DS18B20 (Waterproof)	°C	18.0	22.0	30.0	35.0
Hydrostatic Level	HC-SR04 (Ultrasonic)	cm	20.0	40.0	110.0	130.0
Ambient Temperature	DHT11 / DHT22	°C	5.0	15.0	38.0	45.0
Relative Humidity	DHT11 / DHT22	%	20.0	40.0	85.0	95.0

6.2. Machine Learning Regression Performance

The predictive performance of the Random Forest temperature model was evaluated over a continuous 48-hour dataset sampled at 5-minute intervals ($N = 576$ samples), partitioned into 80% training and 20% out-of-sample test sets.

Table 3. Predictive regression metrics across forward temporal horizons.

Horizon (τ)	Coefficient of Determination (R^2)	MAE (°C)	RMSE (°C)	Inference Latency (ms)
$t + 1$ hour	0.942	0.18	0.24	3.2
$t + 3$ hours	0.891	0.32	0.41	3.4
$t + 6$ hours	0.854	0.47	0.58	3.5

As summarized in Table 3, the model maintains strong predictive fidelity across the 6-hour projection window ($R^2 = 0.854$, $RMSE = 0.58^\circ\text{C}$), providing adequate lead time for automated mitigation (such as activating supplementary aeration or shading) before thermal stress onset.

6.3. Comparative Framework Assessment

Table 4 contrasts AquaMind-AI with existing open-source and commercial aquaculture control systems.

Table 4. Functional benchmarking against existing aquaculture and hydroponic monitoring paradigms.

Feature / Capability	Traditional SCADA	Basic Arduino/IoT	Commercial Cloud	AquaMind-AI
Real-Time Telemetry	Yes	Yes	Yes	Yes (10s interval)
Open Hardware Compatibility	No (Proprietary)	Yes	No	Yes (Plug-and-play)
Horizon Trend Forecasting	No	No	Limited	Yes (Random Forest)
Multivariate Anomaly Isolation	No	No	No	Yes (Isolation Forest)
Autonomous Closed-Loop Control	Rules only	Threshold relay	Cloud-delayed	Yes (Edge & Asynchronous)
Deployment Capital Expenditure	Very High	Low	High (Recurring)	Ultra-Low (< \$60)

7. Regional Implications: Climate-Resilient Agriculture in the Caspian Basin

The South Caucasus and Caspian Basin—notably the arid and semi-arid plains of the Kura-Aras Lowland and Absheron Peninsula in Azerbaijan—experience pronounced climate challenges characterized by rising summer temperatures, high evapotranspiration, and soil salinization.

Conventional agriculture in these areas faces heavy distribution losses and acute seasonal water deficits [7], [8]. Integrating intelligent, recirculating aquaponics via AquaMind-AI offers three major regional benefits:

- **Water Resource Efficiency:** Closed-loop recirculation conserves over 90% of the aqueous medium, requiring freshwater additions only to replace crop transpiration and minimal evaporative losses.
- **Urban and Marginal-Land Food Security:** The system operates independently of soil quality, enabling food production on saline, semi-arid, or degraded land across the Absheron Peninsula, supporting localized production of high-value fish (e.g., Sturgeon, Carp, Tilapia) and vegetable crops.
- **Energy Resilience and Renewable Integration:** The lightweight, asynchronous computing architecture can be powered by off-grid solar PV and battery storage systems, maintaining operations during rural grid fluctuations.

8. Future Trajectories and Industrial Scaling Roadmap

The subsequent developmental phases of the AquaMind-AI platform will focus on three key technical areas:

1. **Multi-Parametric Optical Sensing:** Integrating optical dissolved oxygen (DO) and ion-selective electrode (ISE) arrays with automated drift-compensation algorithms to monitor real-time nitrate (NO_3^-) and ammonium (NH_4^+) ion dynamics.
2. **Edge-AI Computer Vision for Fish Ethology:** Implementing lightweight neural networks (YOLO-based architectures) on edge camera modules (e.g., ESP32-CAM, Raspberry Pi AI) to track swimming velocities, surface gathering behaviors, and feeding response indices.
3. **On-Device TinyML Deployment:** Quantizing Random Forest and Isolation Forest models for direct on-device inference on 32-bit ARM Cortex-M microcontrollers, ensuring continuous local autonomous control during external network outages.

9. Conclusion

This paper presented the comprehensive technical review, system architecture, and empirical validation of AquaMind-AI, an open-architecture cyber-physical platform for smart, climate-resilient aquaponics. By integrating low-cost edge sensors (DS18B20, HC-SR04, DHT11) and optocoupled actuator drivers with an asynchronous FastAPI microservice backend, ensemble Random Forest horizon forecasting, and unsupervised Isolation Forest anomaly detection, the platform provides robust protection against systemic failure modes in closed-loop polyculture systems. The system lowers the technical and economic barriers to smart aquaponics adoption, providing a practical tool for sustainable food production in water-stressed regions.

Author Contributions

Suleyman Hajizade: Conceptualization, Software Architecture, Machine Learning Modeling, Hardware Prototyping, Methodology, Writing – Original Draft, Visualization, Project Administration.

Conflict of Interest

The author(s) declare no conflicts of interest.

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Research Article

Physiological and Biochemical Insights into NADP-Dependent Malate Metabolism under Drought Stress in Bread Wheat

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Abstract

The diverse and essential functions of NADP-MDH and NADP-ME highlight their important roles in carbon allocation and energy metabolism in higher plants. These enzymes provide valuable insights into the biochemical and molecular mechanisms linking photosynthesis, respiration, and photorespiration. Therefore, the activities of two key enzymes involved in malate metabolism, NADP-malate dehydrogenase (NADP-MDH) and NADP-malic enzyme (NADP-ME), were evaluated in the leaves of bread wheat (*Triticum aestivum* L.) genotypes differing in drought tolerance. Among the genotypes studied, the highest NADP-MDH activity was recorded in Murov 2 and Zirva 85. These results indicate that Murov 2 may possess enhanced metabolic adaptation to drought conditions and could serve as a promising genetic resource for breeding programs aimed at developing drought-tolerant wheat varieties, particularly for rainfed and semi-arid environments.

Keywords: drought stress, malate metabolism, NADP-MDH, NADP-ME.

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1. Introduction

In addition to being a period of economic growth, the 21st century also plays an important role in increasing the productivity of cultivated species as a period of intensive study of metabolic processes in plants and the development of methodology in plant research [1]. In the globalized world, the rapid increase in population, the decrease in biodiversity, and the limitation of suitable and productive land areas for agriculture pose a serious threat to meeting people's food needs [2]. In this regard, the direct or indirect effects of abiotic stress factors, including drought, on photosynthesis remain a subject of active discussion among scientists in this field in recent decades [3].

Wheat is an important food product in the world and constitutes 18-20% of human dietary protein [4]. In this context, studying malate dehydrogenase system enzymes with oxidoreductase and decarboxylase activity, which play an important role in increasing wheat productivity, is important [5]. The enzyme NADP-ME reduces NAD(P)⁺ to NAD(P)H in the oxidative decarboxylation of L-malate (MAL) to pyruvate (PYR) [6]. NADP-malic enzyme participates in numerous metabolic processes and plays an important role in plant tolerance to various stress factors. It is known that stress factors such as drought, salinity, and cold affect the entire metabolism by impairing the development of plants. Being a product of the reaction catalyzed by NADP-ME, NADPH, as one of the main factors determining cell development, is involved in the biosynthesis of osmotically active substances such as proline, mannose-6-phosphate, and mannitol. Stress affects the water balance, leading to loss of turgor and membrane breakdown. This condition, caused by stress in the plant cell, requires the regeneration of membrane

lipids. NADP-ME participates in the regeneration process by directing pyruvate and NADPH to the biosynthesis of fatty acids [7].

The NADP-MDH enzyme converts oxaloacetate into malate using excess NADPH to regenerate the NADP electron acceptor. The activity level of the enzyme is higher in the young leaves of the plant and decreases as the leaves age. This fact indicates that the enzymatic activity of NADP-MDH is closely related to active metabolism. The activation of NADP-MDH is inhibited by its product, NADP. Therefore, when NADPH is used for assimilation processes in the chloroplast, NADP-MDH "turns off" its activity. Due to this, the export of reducing equivalents such as malate does not occur. Because ATP formation continues as electrons are transferred to malate, the malate valve plays a crucial role in maintaining the ATP/NADPH balance in chloroplasts as an indirect export system of reducing equivalents.

The study of the regulation mechanisms of metabolic processes creates conditions for understanding the biochemical mechanisms of plant adaptation to stress. From this point of view, the study of malate dehydrogenases and malic enzymes in the active stages of plant development is of particular importance. The study of the effect of soil drought on plant organisms is still of great theoretical and practical importance as an important scientific direction and opens wide prospects for the creation of plant varieties tolerant to unfavorable external environmental factors.

2. Materials and Methods

The genotypes stored in the wheat gene pool of the Research Institute of Crop Husbandry of the Ministry of Agriculture were compared, and 4 genotypes were selected: Murov 2, Aran, Gyzyl bughda, and Zirva 85. Bread wheat genotypes were cultivated in an artificial climate laboratory with a light period of 16/8 hours, 50% relative humidity, and a temperature regime of 24°C/18°C day/night, respectively. Drought stress was applied to 14-day-old seedlings. Measurements were made in 10 biological and 3 technical replicates in 2 variants.

2.1. NADP-MDH activity assay

The determination of the NADP-MDH activity started with the activation of the enzyme. Activation was performed for 15 minutes using 1 M Tris-HCl buffer (pH 8.0) containing 10 mg/ml BSA, 100 mM DTT, and the enzyme preparation. The reaction medium for NADP-MDH was as follows: 100 mM Tris-HCl buffer (pH 8.0), 10 mg/ml BSA, 0.5 M EDTA·4Na, 20 mM MgCl₂, 10 mM NADP-H, and 30 µl activated enzyme preparation. The reaction started by adding 10 µl of 1 mM OA to the reaction medium. The determination of the enzyme activity is based on the decline in optical density for 1 min of the reaction course due to the expenditure of NADH. The millimolar equivalent of ϵ -extinction for NADPH at maximum absorption amounted to 6.22 mM·cm⁻¹ at 340 nm [8].

2.2. NADP-ME activity assay

The enzyme activity was determined based on an increase in the optical density due to the formation of NADPH. The reaction medium for the determination was as follows: 50 mM Tris-HCl (pH 8.2), 1 mM EDTA·4Na, 20 mM MgCl₂, 0.5 mM NADP⁺, 5 mM L-malate·Na and the enzyme extract. The reaction started by adding malate to the reaction medium.

2.3. Relative water content (RWC) Determination

Relative water content (RWC) in leaves was measured based on the method proposed by Turner [9] and calculated using the following formula:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

where: FW – fresh weight of the leaf, TW – turgid weight, DW – dry weight.

Protein was measured by the method of Rekowski, using bovine serum albumin as the standard [10].

The significance of differences between mean values was compared by Student's t-test. Differences at $P \leq 0.05$ were considered significant.

3. Results and Discussion

NADP-MDH is redox-regulated, activated by light, and is inactive in the dark. NADH-MDH, localized in chloroplasts, plays an important role in maintaining a balance of reduced equivalents between cytosol and stroma. Considering this, we determined the NADP-MDH activity in irrigated and drought-exposed variants of chosen wheat genotypes.

The highest NADP-MDH activity was observed in the genotypes Murov 2 and Zirva 85. The enzyme activity changed similarly in the leaves of irrigation and drought variants. The enzyme activity decreased gradually in the leaves of the drought-exposed Gyzył bughda genotype. A decrease in the amount of photosynthetic pigments under long-term soil drought resulted in a gradual decrease in the NADP-MDH activity.

The highest NADP-MDH activity was detected in the genotypes Murov 2 and Zirva 85. After four and six days of irrigation, the enzyme activity increased by 40.0% and 57.2%, respectively. In the leaves of the Zirva 85 genotype, the NADP-MDH activity changed similarly in the irrigated and trial variants. In the leaves of the Gyzył bughda genotype, the enzyme activity decreased gradually under drought (Table 1). The gradual decrease in the amount of photosynthetic enzymes under long-term soil drought caused a gradual decrease in the activity of the light-regulated enzyme NADP-MDH.

Table 1. Dynamics of NADP-MDH activity changes in wheat leaves.

Genotypes	Variants	NADP-MDH activity, EU/mg protein				
		Drought days				
		2	4	6	8	10
Murov 2	irrigation	15.69±1.4	16.71±1.7	17.9±2.0	18.71±1.7	18.9±2.0
	drought	13.04±1.3 ^{ns}	18.3±2.0*	20.5±2.1**	21.7±2.3**	18.9 ±1.8*
Zirva 85	irrigation	18.71±1.4	18.9±2.2	20.71±1.7	24.9±2.7	22.0±2.3
	drought	17.33±1.5*	19.1±2.0**	25.71±1.7*	24.9±2.5**	15.9±1.3*
Aran	irrigation	8.51±0.8	8.9±0.6	10.8±1.2	14.9±1.3	15.9±1.5
	drought	7.1±0.7 ^{ns}	7.9±0.9*	8.7±1.0*	7.9±0.6*	6.7±0.4*
Gyzył bughda	irrigation	13.1±1.8	18.9±1.6	19.8±1.9	24.9±2.3	16.9±1.5
	drought	13.1±1.9**	13.9±1.1*	13.7±1.3*	11.9±1.1*	4.7±0.1 ^{ns}

Note: Results of Student's t – test **, * – significance at the 0.01, 0.05 probability levels, respectively

NADP-ME is involved in many processes related to plant responses to stress at the metabolic level. Thus, the enzyme plays an important role in maintaining the pH of the cytoplasm, regulating the function of the stomatal apparatus, and creating tolerance to stress in plants. Pyruvate, a product of the reaction, is used as a precursor in the biosynthesis of flavonoids and lignin.

The heatmap analysis revealed distinct genotype-dependent patterns in NADP-MDH activity under drought stress. Among the evaluated genotypes, Murov 2 and Zirva 85 exhibited the strongest activation of NADP-MDH during drought exposure. In Murov 2, enzyme activity progressively increased from 13.0 EU mg⁻¹ protein at day 2 to 21.7 EU mg⁻¹ protein at day 8, indicating enhanced metabolic adjustment during prolonged water deficit. Similarly, Zirva 85 showed a marked increase in NADP-MDH activity, reaching the highest value (25.7 EU mg⁻¹ protein) on the sixth day of drought treatment (Figure 1).

In contrast, Aran and Qizil buğda displayed lower NADP-MDH activity throughout the stress period, with a continuous decline observed at later stages of drought. Particularly, Qizil buğda showed a sharp reduction in enzyme activity after 8–10 days of stress, suggesting limited metabolic capacity for maintaining malate turnover under prolonged drought conditions. Overall, the heatmap demonstrates that drought-tolerant genotypes, particularly Murov 2 and Zirva 85, maintain or enhance NADP-MDH activity during water deficit, which may contribute to improved carbon redistribution, redox balance, and metabolic adaptation under drought stress.

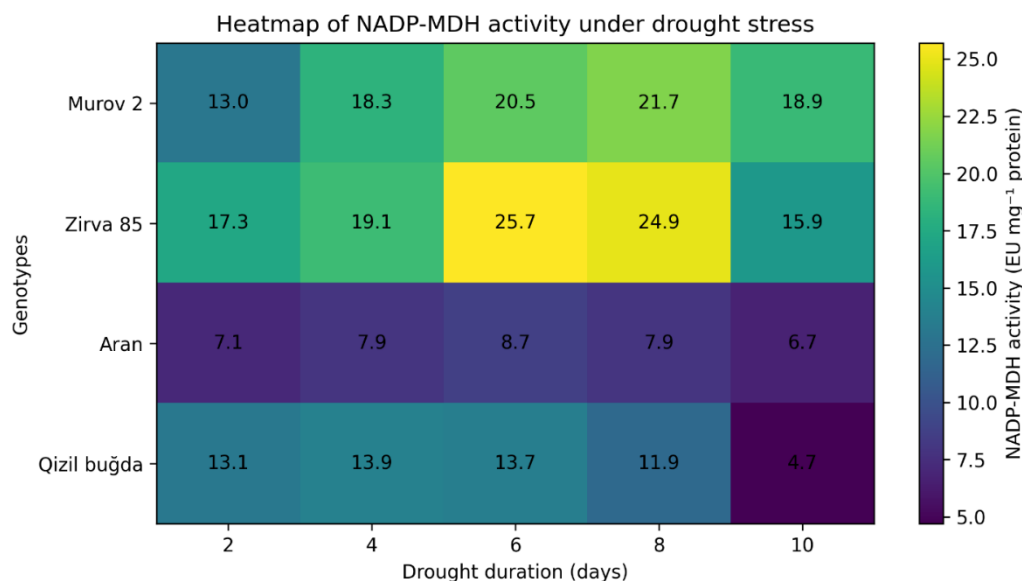


Figure 1. Heatmap visualization of NADP-MDH activity dynamics in bread wheat genotypes under drought stress.

The highest NADP-ME activity was observed in leaves of the Zirva 85 genotype 8 days after irrigation (3.5 ± 0.35 EU/mg protein). In the samples taken from the irrigated variant of the Murov 2 genotype, the activity of the enzyme gradually increased. Significantly lower activity was observed in the leaves of Aran and Gyzył bughda genotypes compared to Murov 2 and Zirva 85 genotypes. The results of our experiments confirm the tolerance of the genotypes Murov 2 and Zirva 85 (Table 2). The responses of plants to water stress differ depending on the plant species, the type of organ and tissue, the compartmentalization of the cell, the intensity and duration of water deficit, and the stage of ontogenesis.

Table 2. Dynamics of changes in NADP-ME activity in wheat leaves.

Genotypes	Variants	NADP-ME activity EU/mg protein				
		Drought days				
		2	4	6	8	10
Murov 2	irrigation	1.38 ± 0.12	1.76 ± 0.18	1.88 ± 0.12	2.27 ± 0.12	2.68 ± 0.12
	drought	$1.24 \pm 0.14^*$	$1.54 \pm 0.16^*$	1.68 ± 0.19^{ns}	2.54 ± 0.25^{ns}	$3.14 \pm 0.36^*$
Zirva 85	irrigation	1.18 ± 0.1	1.3 ± 0.02	1.45 ± 0.15	1.5 ± 0.16	1.8 ± 0.2
	drought	$1.54 \pm 0.18^*$	$1.7 \pm 0.2^*$	$2.45 \pm 0.15^*$	$3.5 \pm 0.35^*$	2.3 ± 0.1^{ns}
Aran	irrigation	0.28 ± 0.02	0.8 ± 0.01	0.84 ± 0.07	1.24 ± 0.05	1.36 ± 0.05
	drought	0.24 ± 0.02^{ns}	$0.44 \pm 0.02^*$	$0.52 \pm 0.02^*$	$0.4 \pm 0.05^*$	$0.76 \pm 0.05^*$
Gyzyl bughda	irrigation	0.31 ± 0.04	0.34 ± 0.04	0.44 ± 0.04	0.74 ± 0.05	1.48 ± 0.05
	drought	$0.24 \pm 0.05^*$	0.36 ± 0.05^{ns}	0.59 ± 0.05^{ns}	$0.24 \pm 0.05^*$	$0.16 \pm 0.05^*$

Note: Results of Student's t – test **, * – significance at the 0.01 and 0.05 probability levels, respectively.

As a result of the increase in temperature, the upper layer of the soil dries up, and osmotic stress occurs around the roots; the concentration of salts and ions that have a toxic effect increases. First of all, this leads to a decrease in the volume and number of leaves [11]. As a result of stress, relative water content in cells and tissues decreases. During drought, due to osmotic adaptation, cells can reduce the osmotic potential and increase the water gradient, maintaining the turgor of the cell by elasticity. This contributes to maintaining the physiological balance of the plant during long-term drought. The weakening of the water storage capacity of plants is more pronounced in drought-sensitive genotypes [12]. Relative water content (RWC) of the leaf, stomatal resistance, transpiration rate, and leaf temperature are important indicators that affect water exchange in the plant. This indicator is high in the

early stages of leaf development and decreases when the dry biomass of the plant begins to increase. The value of RWC in a plant depends on the adsorption of water through the roots and the loss of water through transpiration [13].

Relative water content of the flag leaves of bread wheat genotypes was measured depending on the days of drought in 3 cultivation variants. This parameter was highest in Murov 2 and Aran genotypes, and lower in the Gyzyly bughda genotype compared to others. In the Murov 2 genotype, RWC remained relatively stable. On the 2nd day of the drought, RWC in the control and stress variants was 90% and 89%, and on the 10th day, 85% and 79%, respectively. In the Aran genotype, RWC at the beginning of the drought was 92% in the control variant and 89% in the stress variant, while on the 10th day of the drought, it amounted to 84% in the control variant and 80% in the stress variant. On the 4th day of drought, RWC was 89% in the control variant and 88% in the stress variant of the Murov 2 genotype, while in the Gyzyly bughda genotype, this parameter amounted to 70% and 64%, respectively. Thus, the RWC in the Gyzyly bughda genotype was sharply reduced due to the effect of stress. This can be attributed to shorter roots of the Gyzyly bughda genotype and a relatively stronger osmotic effect (Figure 2).

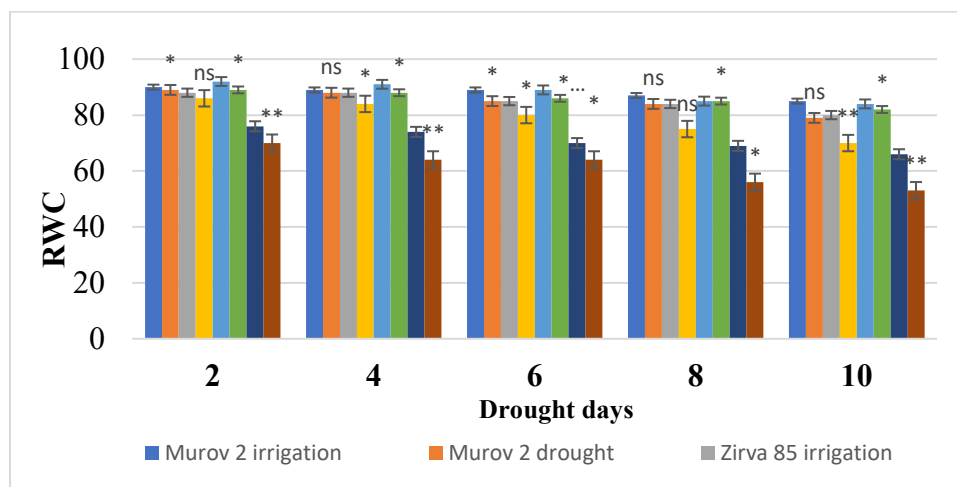


Figure 2. Effect of drought stress on flag leaf relative water content. Each value represents the mean of three replicates.

The relative stability of RWC during drought can be explained by a deeper extension of the root system contributing to less osmotic influence, which has been confirmed by our experiments. NADP-ME participates in the regeneration process by directing pyruvate and NADPH to the biosynthesis of fatty acids. Stress factors that affect water balance also cause oxidative stress by inducing the regeneration of free oxygen radicals. Drought stress, being one of the most common environmental factors that seriously affect plant development and productivity, causes many physiological, biochemical, and molecular changes in plants. Furthermore, the results indicated that organic acids such as malate, oxaloacetic acid, citric acid, and fumaric acid play an important role in the primary carbon metabolism in plants. Thus, the research revealed that the Murov 2 genotype can be cultivated in rainfed and semi-arid regions and can be used as a starting material for creating stress-tolerant wheat varieties in practical breeding programs.

Author Contributions

U. Gurbanova - Conceptualization, Writing; A. Afandiyeva - Methodology, Writing, Submission.

Conflict of Interest

The author(s) declare no conflicts of interest.

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Review Article

Genetic Analysis Of Drought Tolerance and Identification of Candidate Genes in Grapevine (*Vitis Vinifera*) Genotypes

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Abstract

Drought stress is one of the major environmental factors limiting grapevine growth, productivity, and fruit quality, particularly in regions affected by increasing water scarcity and climate variability. Grapevine (*Vitis vinifera*) genotypes exhibit substantial genetic variation in their responses to water deficit, making the identification of genetic factors associated with drought tolerance important for the development of resilient grapevine cultivars. In this review, we discuss the genetic and molecular mechanisms underlying drought tolerance in grapevine, with particular emphasis on genes involved in water regulation, stress signaling, antioxidant defense, osmotic adjustment, and transcriptional regulation. We also examine the potential of molecular markers, gene expression analysis, transcriptomic approaches, and other genomic tools for identifying candidate genes associated with drought-responsive traits. Understanding the genetic basis of drought tolerance may facilitate the identification and characterization of superior grapevine genotypes and support marker-assisted selection and molecular breeding programs. Further investigation of candidate drought-responsive genes could contribute to the development of grapevine cultivars with improved adaptation to water-limited environments and help maintain grape production under changing climatic conditions.

Keywords: grapevine, drought tolerance, drought stress, genetic diversity, candidate genes, plant genetics, molecular markers.

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1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most economically important perennial fruit crops worldwide and is cultivated extensively for wine, table grapes, juice, and dried fruit production. Because grapevine cultivation is strongly influenced by environmental conditions, the availability of water is a major factor determining plant growth, productivity, and fruit quality. Many grape-producing regions are characterized by warm and dry growing seasons, and increasing temperatures together with irregular precipitation patterns are expected to increase the frequency and severity of drought events. Consequently, water scarcity has become an important challenge for sustainable viticulture, particularly in regions where irrigation resources are limited [1], [2].

Drought stress affects grapevines at multiple physiological and biochemical levels. A reduction in soil water availability can alter stomatal conductance, photosynthesis, water transport, osmotic regulation, and antioxidant activity, ultimately affecting vegetative growth and berry development. Grapevine cultivars also differ considerably in their responses to water deficit, indicating that drought adaptation is influenced by genotype as well as environmental conditions. Differences among genotypes have been associated with traits such as stomatal regulation, hydraulic conductivity, root characteristics, aquaporin activity, abscisic acid (ABA) dynamics, and osmotic adjustment [1].

The molecular response of grapevine to drought involves complex regulatory networks rather than a single physiological mechanism. ABA-mediated signaling has an important role in the response to water deficit by regulating processes associated with stomatal closure and plant water balance. In addition, drought stress can induce changes in the expression of genes involved in hormone signaling, reactive oxygen species (ROS) metabolism, osmotic adjustment, carbohydrate metabolism, and cellular protection [2].

Transcriptomic investigations have further demonstrated that grapevine genotypes can activate distinct sets of genes under water-limited conditions, suggesting that genotype-dependent molecular responses contribute substantially to differences in drought adaptation [3].

The identification of genes associated with drought-responsive traits is therefore an important component of understanding the genetic basis of drought tolerance in grapevine. Advances in molecular genetics and genomics have provided several approaches for investigating this genetic basis, including molecular markers, gene expression analysis, transcriptomics, genome-wide association studies (GWAS), and comparative genomic analyses. For example, a GWAS study involving different *Vitis* species identified significant marker-trait associations related to stomatal conductance during progressive water deficit and identified candidate genes potentially involved in drought response. The study demonstrated that genetic variation can be associated with physiological traits that contribute to water-use regulation in grapevine [4].

2. Physiological and Biochemical Responses of Grapevine to Drought Stress

2.1. Water Relations, Stomatal Regulation and Photosynthesis

Water availability strongly influences the physiological performance of grapevine and determines how effectively the plant can maintain growth and carbon assimilation during periods of drought. When soil moisture declines, the movement of water from the soil into the plant becomes increasingly restricted, creating changes in leaf water status and hydraulic functioning. Grapevines respond to these changes through coordinated adjustments in water uptake, xylem transport, transpiration, and stomatal behavior. The interaction between these processes determines the capacity of a plant to maintain water balance under prolonged water limitation [5], [6]. Under water deficit, grapevines reduce stomatal conductance and transpiration to limit water loss [7]. The intensity of this response varies among genotypes and is influenced by hydraulic properties and ABA signaling [8]. Grapevine genotypes may adopt contrasting isohydric or anisohydric strategies under drought, involving differences in stomatal regulation, ABA response, xylem pH, and hydraulic adjustment. Stomatal closure also restricts CO₂ entry into leaves, resulting in reduced photosynthetic activity [9], [10]. Under moderate drought, photosynthesis can be maintained despite partial stomatal closure, whereas severe drought causes additional limitations in carbon fixation and photosynthetic processes [11]. Therefore, differences in stomatal regulation and photosynthetic maintenance among genotypes can be useful for identifying drought-tolerant grapevine varieties [5], [7].

2.2. Oxidative Stress and Antioxidant Defense

Drought stress disrupts cellular redox balance and promotes the accumulation of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide [5]. Excessive ROS can damage lipids, proteins, and nucleic acids, whereas controlled ROS production can also function as a signaling mechanism during stress adaptation. The strength of this antioxidant response differs among grapevine genotypes and can contribute to their contrasting levels of drought tolerance [12]. Recent evidence also indicates that drought-responsive transcription factors such as VvNAC33 can enhance grapevine drought tolerance by promoting antioxidant activity and ROS detoxification [13].

Grapevine possesses a complex antioxidant network that helps maintain cellular redox balance during drought stress [13]. This network includes enzymatic components such as superoxide dismutase, catalase, and peroxidases, together with non-enzymatic antioxidants including ascorbate and glutathione [14]. The activity of these protective systems may vary between grapevine genotypes, which can partly explain differences in their ability to tolerate water deficit [15]. In addition, changes in the expression of antioxidant-related genes can contribute to the regulation of ROS levels during drought [16]. The coordinated activation of these defense mechanisms helps

reduce oxidative damage and supports the maintenance of cellular functions under prolonged water limitation [13], [16].

3. Molecular Mechanisms of Drought Tolerance

3.1. ABA Signaling and Drought-Responsive Pathways

Abscisic acid (ABA) is a major hormonal regulator of grapevine responses to drought stress. Water deficit promotes ABA accumulation, which contributes to stomatal regulation and activation of stress-responsive genes. As water availability decreases, increased ABA signaling helps the plant coordinate water conservation with broader cellular responses to drought. This regulation is particularly important because stomatal closure reduces excessive water loss while simultaneously influencing CO₂ uptake and photosynthetic activity [2]. At the molecular level, ABA is perceived by PYR/PYL/RCAR receptors, which inhibit PP2C and allow SnRK2 kinases to become active. The activation of this signaling cascade provides a molecular connection between the perception of water deficit and the regulation of downstream stress responses [17]. Activated SnRK2 proteins subsequently regulate downstream transcription factors, including ABF/AREB proteins, leading to the expression of drought-responsive genes [2], [17]. Through this pathway, ABA signaling can influence several physiological processes associated with drought adaptation, including stomatal behaviour, cellular protection and regulation of stress-related gene expression. Studies in *Vitis vinifera* have identified multiple components of this signaling pathway, including PYR/PYL receptors, PP2Cs, SnRK2s and ABFs [17]. Differences in the expression of ABA-related genes among grapevine genotypes may contribute to variation in drought tolerance [18]. Such differences may affect the intensity and timing of ABA-mediated responses and consequently influence how individual genotypes respond to prolonged water limitation,

3.2. Osmotic Adjustment, Aquaporins and Stress-Responsive Genes

Drought causes a decline in cellular water availability, and grapevine can partially compensate for this change through osmotic adjustment. Grapevines can maintain cellular water balance during drought by accumulating compatible solutes and mineral ions, which support the preservation of cell turgor under low water availability [1]. Among these compounds, proline, soluble sugars, potassium, and calcium have been associated with osmotic regulation, although their contribution may vary depending on the genotype. The accumulation of these compounds can help cells retain water and maintain metabolic activity when external water availability becomes limited. Osmotic adjustment is therefore an important mechanism that allows grapevine tissues to tolerate changes in cellular water potential. Among these compounds, proline, soluble sugars, potassium, and calcium have been associated with osmotic regulation, although their contribution may vary depending on the genotype.

In addition, drought activates numerous stress-responsive genes involved in ABA signaling, osmotic regulation, antioxidant defense, and carbohydrate metabolism, demonstrating that grapevine drought tolerance is controlled by a complex molecular network [3]. These genes participate in different stages of the stress response and allow grapevine cells to coordinate water conservation with cellular protection. Changes in carbohydrate metabolism can also influence the availability of soluble sugars that contribute to osmotic adjustment. At the same time, activation of antioxidant-related genes helps protect cells from oxidative damage associated with drought stress. The expression of these stress-responsive genes may differ between grapevine genotypes, suggesting that genetic variation contributes to differences in drought adaptation. Overall, osmotic adjustment, aquaporin regulation, and stress-responsive gene expression function as interconnected mechanisms that help grapevine maintain cellular stability under water-limited conditions [1], [3].

4. Identification of Candidate Genes Associated with Drought Tolerance

4.1. Transcriptomic and Gene Expression Approaches

Transcriptomic methods, particularly RNA-sequencing, are widely used to investigate grapevine responses to drought stress. These approaches allow researchers to identify differentially expressed genes involved in ABA

signaling, carbohydrate metabolism, antioxidant defense, and stress regulation [3], [19]. Gene expression patterns can vary among grapevine cultivars, providing insights into their different drought-adaptation strategies [15]. Therefore, transcriptomic analysis is useful for identifying candidate genes and molecular pathways associated with drought tolerance [19].

RNA-sequencing also makes it possible to examine large numbers of genes simultaneously, providing a broader view of the molecular response to water deficit. Changes in transcript abundance can reveal which biological processes are activated or suppressed when grapevines experience drought conditions. This information can be particularly useful for distinguishing early responses from changes that occur after prolonged stress. Transcriptomic comparisons between stressed and well-watered plants can therefore provide information about the molecular reprogramming that accompanies drought exposure [3], [19]. The analysis of differentially expressed genes can identify functional groups associated with stress perception, signal transduction, and cellular protection. Genes related to transcriptional regulation may also play an important role because transcription factors can coordinate the expression of multiple drought-responsive genes. In addition, alterations in genes involved in carbohydrate metabolism may reflect changes in carbon allocation and osmotic regulation during water limitation. Similarly, changes in antioxidant-related transcripts can indicate the activation of mechanisms responsible for controlling oxidative stress [3], [15].

An important advantage of transcriptomic analysis is that it can reveal differences between grapevine cultivars exposed to the same environmental conditions. Some cultivars may activate particular stress-related pathways more strongly or at an earlier stage than others, contributing to their contrasting drought responses [15]. Such genotype-dependent expression patterns can help explain why grapevine varieties differ in their ability to maintain physiological activity during water deficit. Transcriptomic data can also be combined with physiological measurements to establish relationships between gene expression and traits such as stomatal conductance, photosynthesis, and water-use efficiency.

Furthermore, transcriptomic studies can provide a starting point for identifying candidate genes that require further functional investigation. Genes showing consistent associations with drought-related responses may subsequently be examined using molecular and genetic approaches. The integration of transcriptomic analysis provides an important molecular framework for studying grapevine drought responses and identifying biological ways that may contribute to the development of more drought-tolerant genotypes.

4.2. Molecular Markers, GWAS and Candidate Gene Identification

Molecular markers are valuable tools for identifying genomic regions associated with drought tolerance in grapevine. Techniques such as SSR and SNP markers, together with genome-wide association studies (GWAS), enable the detection of marker-trait associations linked to physiological responses under water deficit. GWAS analyses have identified several candidate genes involved in stomatal regulation, water transport, and stress signaling, providing important targets for marker-assisted breeding of drought-tolerant grapevine genotypes [4]. GWAS can also narrow the search for genes located within genomic regions associated with drought-related characteristics.

5. Application of Genetic Knowledge in Grapevine Breeding

5.1. Identification and Selection of Drought-Tolerant Genotypes

The identification of drought-tolerant grapevine genotypes requires the evaluation of several physiological and agronomic characteristics rather than relying on a single trait. Parameters such as stomatal conductance, water-use efficiency, photosynthetic performance, yield, and root characteristics can help distinguish genotypes with better adaptation to water deficit [20]. Because drought responses are strongly influenced by environmental conditions, combining multiple traits provides a more reliable basis for selecting promising genotypes. Such screening can support the selection of suitable cultivars and rootstocks for breeding programs aimed at improving grapevine drought tolerance.

5.2. Molecular Breeding and Future Biotechnological Approaches

Molecular breeding can accelerate the improvement of drought tolerance by combining genetic markers, genomic information, and physiological traits during selection [20]. Genome-editing technologies, particularly CRISPR/Cas systems, provide an additional approach for investigating and modifying genes involved in drought-response pathways [20]. These technologies may help develop grapevine genotypes with improved water-use efficiency and stress adaptation while reducing the time required for conventional breeding. The integration of genomic selection, transcriptomic information, and gene editing is therefore a promising direction for future grapevine improvement.

6. Conclusion

Drought stress represents one of the major environmental challenges affecting grapevine growth, productivity, and long-term adaptation, particularly under changing climatic conditions. The response of grapevine to water limitation involves a complex interaction between physiological, biochemical, and molecular mechanisms. Regulation of stomatal conductance and photosynthesis allows plants to balance carbon assimilation with water conservation, while antioxidant systems help protect cells from oxidative damage caused by excessive reactive oxygen species. At the molecular level, ABA signaling, osmotic adjustment, and drought-responsive genes contribute to the coordination of these responses.

Genetic and transcriptomic approaches provide further opportunities to understand the mechanisms underlying differences in drought tolerance among grapevine genotypes. RNA-sequencing, molecular markers, and GWAS can facilitate the identification of candidate genes and genomic regions associated with important drought-related traits. Combining these molecular approaches with physiological screening may improve the selection of genotypes capable of maintaining water status, photosynthetic activity, and cellular stability under water deficit. Such integration is particularly valuable for grapevine improvement because drought tolerance is controlled by multiple interacting traits rather than by a single gene or mechanism.

Overall, integrating traditional genetic approaches with modern biotechnology can contribute to more efficient grapevine breeding and support sustainable grape production under increasingly limited water availability. A more comprehensive approach to grapevine improvement can be achieved when DNA-based marker data are interpreted alongside ampelographic observations. Such integration allows for more accurate cultivar identification and supports the selection of genotypes that combine stress tolerance with desirable agronomic and enological traits. Moreover, combining molecular fingerprinting with morphological descriptors enhances the conservation and sustainable utilization of genetic resources, providing a robust framework for germplasm management in breeding programs [21].

Future research should therefore focus on combining physiological measurements, genomic information, and functional gene analysis to obtain a more comprehensive understanding of grapevine drought adaptation. Molecular breeding, genomic selection, and genome-editing technologies may provide additional opportunities to accelerate the development of drought-resilient grapevine varieties. Overall, integrating traditional genetic approaches with modern biotechnology can contribute to more efficient grapevine breeding and support sustainable grape production under increasingly limited water availability.

Author Contributions

The author conceived and designed the study, conducted the research, analyzed the data, and wrote the manuscript.

Conflict of Interest

The author(s) declare no conflicts of interest.

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Review Article

Common Diseases Frequently Encountered in Fish of the Order Cypriniformes, Control Measures and Prevention

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Abstract

This article investigates the diversity of fish belonging to the order Cypriniformes, their main biological characteristics, and various diseases affecting them. The main aim of the study is to provide a general characterization of fish species belonging to the order Cypriniformes, determine their distribution patterns and economic importance, and investigate the parasitic, viral, bacterial, and fungal diseases occurring in these fish. The first chapter presents the general characteristics and systematic position of the order Cypriniformes, as well as information about its major representatives. Their diversity and biological characteristics are also analyzed. The second chapter examines the main diseases observed in fish belonging to the order Cypriniformes. Information is provided on the causes, causative agents, transmission routes, and effects of these diseases on fish health. In addition, the characteristics of parasitic, viral, bacterial, and fungal diseases, their economic impact on fish farming, and measures for disease control are discussed. Based on the results of the study, it was determined that the implementation of preventive measures is of great importance for protecting fish belonging to the order Cypriniformes from diseases, detecting diseases at an early stage, and preventing their spread. The article also provides recommendations concerning compliance with sanitary and hygienic requirements in fish farms, monitoring water quality, regular veterinary supervision, and the timely implementation of disease control measures.

Keywords: Cypriniformes, fish, species diversity, parasitic diseases, viral diseases, bacterial diseases, fungal diseases, prevention, fish farming.

1. Introduction

As integral components of aquatic ecosystems, fish play a crucial role in preserving biodiversity and ensuring food security, economic development, and social well-being—factors of strategic importance to human societies. Their importance, particularly as a source of high-protein food, has grown; consequently, aquaculture and commercial fishing have become significant economic sectors in many countries, including Azerbaijan. In this context, cyprinid fish belonging to the order Cypriniformes—such as common carp, bream, kutum, and other species—are regarded as commercially viable species that are widely cultivated in aquaculture. Their high adaptability, feed conversion efficiency, and reproductive performance ensure successful rearing in both natural and artificial environments (lakes, reservoirs, and breeding farms). However, the intensification of the aquaculture sector, high population densities, inadequate zoohygienic conditions, and deteriorating water quality have increased the prevalence of infectious diseases of various origins among cyprinid fish. In addition to causing reduced productivity and economic losses, these diseases can also disrupt the ecological balance in water bodies. Therefore, the study and prevention of infectious diseases in catfish remain relevant from both scientific and practical perspectives [1].

The primary objective of this study is to investigate infectious diseases affecting cyprinid fish (Cypriniformes), their causative agents, and the conditions of their spread; to identify clinical manifestations and key risk factors and to analyze modern preventive and therapeutic measures on a scientific basis. The primary objective of this study is to investigate infectious diseases affecting cyprinid fish (Cypriniformes), their causative agents, and the conditions of their spread, to identify clinical manifestations and key risk factors, and to analyze modern preventive and therapeutic measures on a scientific basis, to provide a general classification of bacterial, viral, parasitic, and fungal diseases found in catfish-like fish; to analyze the most common disease-causing agents (*Aeromonas hydrophila*, KHV, *Ichthyophthirius multifiliis*, etc.) and their biological characteristics; to identify the key environmental and technogenic factors influencing the spread of diseases (water temperature, oxygen content, ammonia levels, etc.); to summarize existing scientific information on the diagnosis, clinical signs, and treatment methods of diseases; to investigate modern preventive approaches, including the potential application of molecular diagnostics, vaccination, and alternative treatment methods.

2. Materials and Methods

Analytical and scientific research methods based on various sources were employed in conducting the research. Interrelationships between the causes and consequences of various diseases have been established, and scientific findings have been synthesized. Mechanisms of action and routes of transmission were compared across different disease groups (bacterial, viral, parasitic, fungal). The effectiveness of modern preventive and therapeutic methods (probiotics, plant-based antimicrobial agents, etc.) was evaluated through analysis.

3. Results and Discussion

This article is a study dedicated to a detailed scientific analysis of diseases affecting sturgeon-like fish, systematically summarizing the biological characteristics of the causative agents, clinical manifestations, and conditions of spread. Unlike previous studies, this work investigates diseases of diverse origins collectively and presents a comprehensive overview of modern diagnostic and therapeutic approaches. In addition, the transmission mechanisms and risk factors of certain diseases (e.g., KHV and SVC) have been analyzed within the context of local conditions. At the same time, information regarding the potential application of plant-based antimicrobial agents, probiotics, and alternative preventive approaches has been summarized in such detail for the first time within the context of Azerbaijan.

The theoretical significance of the study lies in the systematic scientific investigation of etiology, clinical presentation, causes of spread, and treatment methods of diseases affecting sturgeon-like fish. This establishes a solid theoretical foundation for future scientific research by creating a reliable database for researchers in the fields of biology and veterinary science. The practical significance of the research may prove beneficial for specialists working in water management, veterinarians, and managers of aquaculture enterprises. The diagnostic methods, treatment measures, and preventive approaches outlined here offer practical opportunities for the timely prevention of diseases, the maintenance of healthy fish populations, and the enhancement of farm productivity. Thus, this research can contribute to the sustainable and sound management of aquatic bioresources, both scientifically and in the practical sphere.

3.1. Species Diversity of Cyprinids

The order Cypriniformes is one of the most widely distributed and species-rich groups among bony vertebrates. Fish belonging to this group primarily inhabit freshwater environments and account for approximately 80% of the world's freshwater fauna. Although their natural distribution ranges are largely confined to Eurasia and North America, certain species have subsequently spread to other regions due to anthropogenic influences [2].

The Cyprinidae family is considered the largest among freshwater fish in terms of the number of species. In these fish, the scales are small and cycloid in structure. In some representatives, however, scales are absent, and the skin is naked. The length of most members of the Cyprinidae family ranges from 20 to 40 cm. Among some of the

larger species such as grass carp and thicklip grey mullet. There are various ecological groups of catfish based on their habitat. Semi-anadromous, migratory, and freshwater species are the most widely distributed among these ecological groups. Thus, common carp, bream, roach, kutum, and Caspian roach are classified as semi-anadromous species; shemaya, asp, and poru as anadromous species; while broadhead, rudd, topmouth gudgeon, gudgeons, khramulya, golden nase, and black bream are freshwater species. Freshwater species themselves are divided into two groups: rheophilic and limnophilic species. Rheophilic fish travel to flowing water to spawn. Examples of these include gudgeons, loaches, golden nase, and naked loaches. Limnophilic species, on the other hand, do not require flowing water for spawning and lay their eggs directly in their usual habitat. Examples of these include broadhead, rudd, mudfish, tench, and perch [2].

Carp also vary in their feeding habits. Shemaya and silver bream mainly feed on plankton; asp, being a predatory species, feeds on fish and other aquatic organisms smaller than itself; khramulya and topmouth gudgeon feed on detritus; common carp, bream, and roach feed on benthic organisms; grass carp feeds on higher aquatic plants; while thicklip grey mullet feeds on both lower and higher algae [2], [3].

Several groups of Cypriniformes are distinguished based on their reproductive habits. These include phytophiles, lithophiles, pelagophiles, and ostracophiles. One of the distinctive and interesting characteristics of carp species, compared with other fish, is the occurrence of interspecific fertilization. New, hybrid forms arise during this type of fertilization. The most widespread examples of interspecific fertilization are the hybridization of kutum with roach and the hybridization of roach with bream. Such fish are of considerable importance to fisheries because this type of hybridization produces more resilient hybrid forms. The family currently comprises 220 genera and 2,420 species [2].

3.2. Parasitic diseases of fish belonging to the Cyprinidae family

Parasites found in freshwater and saltwater fish spend part or all their lives in these organisms and act as pathogenic agents of disease in the fish. Since natural aquatic environments cover vast areas and fish populations are widely distributed, it very rarely becomes pathogenic to fish in natural waters. However, they frequently cause diseases in agricultural enterprises and lead to significant financial losses. Fish parasites are unicellular (protozoa) and multicellular (metazoa) organisms. They are found as ectoparasites (external parasites) on the skin, gills, and fins, and as endoparasites (internal parasites) in internal organs and tissues. While some are indirect causes of disease, many are considered direct pathogens for fish. However, parasitic diseases make fish susceptible to other pathogens (bacteria, viruses, and fungi). Their biology is straightforward; the life cycles of some include fish or small aquatic organisms (typically invertebrates). Furthermore, certain parasites found in fish manifest as zoonoses in humans and cause various diseases [4], [5].

Ichthyobodosis

Agent: *Ichthyobodo necator*. It is a primitive unicellular (protozoan) organism. The areas it inhabits are the gills, skin, and particularly the dorsal fins in fish. It has a worldwide distribution. The parasite, which is more common in fish fry, can cause disease in fish of all age groups. Although the disease occurs in every season, fish are more prone to it during the summer and autumn months [5]. It causes hyperplasia in the epithelium and the formation of dark-colored spots on the skin. These areas of the skin appear grayish. Areas of scaling and minor erosion develop symmetrically on both sides of the dorsal fin. Increased mucus production, erosion, and fraying occur on the fins. Loss of appetite, weakness, and sluggish movement are observed in sick fish. Although the disease occurs throughout the year, severe infections are most frequently encountered in late spring and early autumn. If the agents are present in the aquatic environment, the first symptoms in fish begin to appear after two weeks. In infected fish, parasites are predominantly found on the skin surface. If parasites are not removed from the water, a monthly fish mortality rate of 1% in the facilities is inevitable [5].

Anisakiasis

Agent is a helminth belonging to the genus *Anisakis*. They include *Anisakis simplex*, *Pseudoterranova decipiens*, *Contracaecum osculatum*, and *Anisakis pegreffii*. They develop in various species of fish and mammals within the aquatic food chain. It is found in the digestive system, muscles, and on the surface of internal organs of marine

fish, particularly cyprinids [6]. In infected fish, rapid weight loss and mortality may be observed because of tissue destruction in the liver, pyloric caeca, and blood vessels caused by the parasite. The disease occurs when humans accidentally consume raw or undercooked fish (sushi, sashimi, etc.) infected with these parasites. Infection caused by anisakid larvae is generally confined to the stomach and intestines, but in some cases, it can be invasive and cause allergic symptoms, nausea, vomiting, diarrhea, abdominal pain, and gastrointestinal discomfort in humans. It has been established that freezing fish at -20°C for 24 hours, cooking it (to an internal temperature of 76°C), or salting it for more than 24 hours is sufficient to kill anisakids [6].

Diphylllobothriasis

Agent: They are tape-like helminth worms belonging to the genus *Diphylllobothrium*. The adult form lives in the small intestines of fish-eating mammals (cats, dogs, foxes) and birds, as well as humans. *Diphylllobothrium dendriticum* and *D. ditremum* plerocercoids inhabit various locations in salmonid fish—such as the body cavity wall, muscles, liver, and the surface of reproductive organs—and are found within cysts (resembling pearls). The *D. latum* plerocercoid migrates into muscle tissue but does not reside in cysts; it is typically found lying freely beneath the skin [7]. It can result in weakness, weight loss, stunted growth, swimming near the water's surface, and even fish death. In addition to causing liver damage, plerocercoids also lead to abdominal swelling in fish. Severe muscle degeneration sometimes occurs; the fish's head twists to one side due to spasm, and the dead fish assume an "S" shape [7].

3.3. Viral diseases affecting fish of the Cyprinidae family

Spring viraemia of carp (SVC)

The agent, which has a very wide distribution range in Europe, belongs to the genus *Vesiculovirus* of the family Rhabdoviridae. This is a highly contagious and serious infection that causes disease in carp worldwide. The SVC virus was listed as a notifiable pathogen in the list published by the OIE in 2011. The virus is sensitive to ether, heat, and acid. The optimal pH for replication is 7.0–10.0. While the mortality rate is significantly high during the spring months—when water temperatures range from 10°C to 17°C —at higher temperatures, the humoral antibodies produced by infected fish can halt the virus's spread and protect against reinfection [8]. The agent causing the fatal infection shows an affinity for capillary endothelial cells, nephrons, and hematopoietic structures. The clinical manifestations of the virus in the tissues are generally hemorrhagic formations and tumors. The congregation of fish at the pond inlets in production facilities can be considered an early sign of SVC. Signs include unbalanced and erratic swimming behavior and an acidic, bloody fluid discharge from the anal region of the fish when removed from the water [9]. Although the SVC virus can infect other freshwater fish, its primary impact is on carp (*C. carpio*). External examination may reveal hemorrhages in the skin and eyes, exophthalmos, and abdominal distension; internal examination may show peritonitis, ascites, catarrhal and hemorrhagic enteritis, and hemorrhages in the skeletal muscles and swim bladder walls. The agent was also isolated from the kidneys and spleen of infected fish [8], [9]. The infection can be diagnosed based on clinical findings; additionally, the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are used for isolation and identification. To protect against the pathogen, fish farms infected with the disease must be placed under quarantine, and the movement of fish in the area must be halted. In our country, this is a notifiable disease under the Law on Veterinary Medicine.

Koi Herpesvirus Disease (KHVD)

The agent is Cyprinid Herpesvirus-3 (CyHV-3), a species of the genus *Alloherpesvirus* within the Herpesviridae superfamily. Sequence analyses show that CyHP-3 is closely related to the agents CyHP-1 and CyHP-2. Although mass mortality events caused by the koi virus were first reported in the USA and Israel in 1998, sample analyses indicated that the agent had been present in wild carp in Great Britain since 1996. Today, CyHV-3 is found in the rest of the world, except for South America, North Africa, and Australia. CyHV-3 is transmitted through direct contact with infected fish, as well as via the body fluids of infected fish, contaminated mud, water, and so on. The agent primarily enters the fish through the skin. KHV infection typically occurs during the spring months when the water temperature is between 16°C and 25°C , and the incubation period ranges from 7 to 21 days, depending on the water temperature. KHV disease, which has a 100% morbidity rate and an 80–90% mortality rate and can

affect carp of all ages, can cause inflammation, hyperplasia, and necrosis in the fish's gills. The organs most affected during KHV infection include the fins, kidneys, and spleen. Diagnosis of the infection can be made by examining typical clinical findings. However, histological examination reveals large-cell proliferation in the epithelium. Although the agent was isolated through inoculation of KF-1 cell cultures, the PCR method is far more effective than this technique. Although a safe and effective vaccine is not currently available, attenuated vaccines can be used to some extent in protecting fish. Although the vaccine stimulates the production of antibodies against the virus, the duration of protection it provides is uncertain. Based on a 2009 OIE study, it was observed that the local administration of liposome-based vaccines containing inactivated KHV protects against infection [10], [11].

3.4. Bacterial diseases affecting fish of the Siluridae family

Furunculosis

This is an infection characterized by the formation of furuncles on the bodies of fish and a septicemic course. It is quite widespread. The causative agent of the disease is *Aeromonas salmonicida*. Rainbow trout (*Oncorhynchus mykiss*) are resistant to this infection. This bacterium is one of the oldest known fish pathogens [12]. Furunculosis in fish manifests in acute, subacute, and chronic forms. The appearance of furuncles on the bodies of infected fish is a characteristic symptom. The incubation period of the disease varies depending on the number of bacteria ingested by the fish, the route of entry into the body, and environmental conditions. Acute cases are observed primarily in fish fry. The incubation period is very short. Deaths among the animals begin within 2–3 days, without any clinical signs. In the fish, lethargy, bleeding from the nostrils, hemorrhages on the body surface and fins, and numerous small lesions between the subcutaneous tissue and muscles occur. Boils on the body typically appear in large fish suffering from chronic disease; when these lesions rupture, they form ulcers with raised edges that discharge purulent, microbe-laden fluid [13].

Motile Aeromonas infections

Motile *Aeromonas* species cause significant hemorrhagic septicemia in freshwater fish. Aeromonads are characteristic freshwater bacteria. Due to their mobility, they are frequently encountered in the ecosystem. They are found in water and its microflora, as well as in various aquatic organisms, and are pathogenic to humans [14]. Among the motile *Aeromonas* species that cause infections in fish, the most common infections are caused by *A. hydrophila*. *A. hydrophila* has been identified as a pathogen in cyprinids, reptiles, amphibians, invertebrates, and many mammals, as well as in other marine and freshwater fish. When *Aeromonas* infections occur in fish alongside parasitic infections under unfavorable conditions, they frequently cause inflammation and lesions on the fish's skin. These lesions first appear on the fish's skin and gills, then spread to the muscles and subsequently to all internal organs, and their treatment takes a long time. Aeromonads cause infections in small ponds and lakes, particularly in colorful ornamental fish with high body resistance, and most frequently in carp [15].

Fungal diseases in fish belonging to the Cobitidae family

Aquatic fungal agents (eggs, zoospores, adult forms, etc.) cause diseases and mortality in fish at various stages of development. Fungal infections usually appear as secondary infections; by colonizing lesions caused by primary agents (bacteria, viruses, parasites), they alter the course of the infection. Even if fungal pathogens are present in the water, fungal diseases do not develop if the fish is healthy, there are no skin injuries, and the protective mucous layer on the skin is intact. Most aquatic fungi are saprophytes and sustain themselves by decomposing organic matter. When a fish sustains mechanical injury or contracts another infection, a fungal agent often infects the fish secondarily [16].

Saprolegniosis

It is an external fungal infection frequently found in freshwater fish (especially trout and carp), marine fish, and fish eggs. It inhabits the head, eyes, mouth, gills, skin, and eggs of fish living in warm and cold waters. It occurs particularly in carp farming facilities. Although information is available on many species, the most widespread and well-known species are *Saprolegnia parasitica* and *S. invaderis* [17]. Clinically, lesions consisting of grayish-white patches on the skin are evident, caused by fungal spores and cotton-like, woolly structures extending outward from the body. The most noticeable initial sign is the appearance of white, cotton-like growths on the fish's skin

and gills. Depending on the mycelium's ability to trap sediments or algae from the water, the color of these lesions may appear red, brown, or green. Because the fungal mycelium grows not only in the skin but also in the subcutaneous and muscle layers, it causes necrosis in the tissues. If the mycelium covers 30–40% of the body surface, treatment is unsuccessful. Sometimes the infection spreads to the esophagus, while in other cases, it extends to other tissues and organs such as the liver, spleen, kidneys, swim bladder, eyes, and brain. Lethargy, loss of appetite, and loss of balance are observed in the fish, while the eggs exhibit an opaque appearance. In general, extensive skin necrosis, swelling, and hemorrhage are observed in the muscle tissue. In some cases, the starting point of the infection is the digestive system. In infections originating from the digestive system, hyphae developing in the stomach penetrate the stomach wall, muscle layer, and skin, growing outward; mycelial lesions are observed in the ventral region of the stomach during this process. A fungal infection has been detected in the internal organs [18].

Branchiomycosis

It is an acute fungal disease localized exclusively in the gills of freshwater fish. They are observed in temperate regions. They are in the capillaries as well as in the epithelium. In this infection, which is most frequently observed in carp and trout, the most common and well-known species are *Branchiomyces demigrans* (tuna, perch, rudd) and *B. sanguinis* (carp, rudd) [19]. Although the pathogenicity of both species is similar, *Branchiomyces demigrans* appears in the epithelium and blood vessels, whereas *B. sanguinis* is found on the inner surface of the gills. While *Branchiomyces demigrans* destroys epithelial tissue and proliferates externally, *B. sanguinis* develops solely within the blood vessels of the gills and does not tend to grow externally. As they cause respiratory distress, the most common signs observed in fish during subacute and acute infections are difficulty breathing or lethargy. This situation is typically observed in temperate regions during the summer months, when water temperatures exceed 20°C, and the mortality rate reaches 650. The disease rate can reach 100%, but not all fish have the same susceptibility. The progression of the infection is generally rapid and can worsen under conditions of overcrowding, algal blooms, and rising levels of un-ionized ammonia [20]. Complications in humans are caused by diseases affecting crustaceans, along with control measures and prevention strategies.

4. Complications in humans caused by diseases affecting crustaceans, along with control measures and prevention strategies

Fish play an active role in causing food poisoning, accompanied by diarrhea in humans. This situation leads to a loss of public confidence in these products and a decline in the demand for their consumption. Mussels and oysters are the primary food sources associated with enteritis, as their ability to filter-feed causes them to accumulate large quantities of microbes from sewage-contaminated water. Even if these foods contaminated with sewage-borne microorganisms are thoroughly cooked, pathogens within the internal organs may not be sufficiently destroyed. In this process, trace amounts of intestinal matter may remain in the food, and these substances cause illness after consumption [21].

Some bacteria pathogenic to humans are abundant in natural waters. These bacteria generally belong to the *Vibrio* and *Aeromonas* groups [22]. High levels of these agents in seafood pose a threat to public health. In seafood processing plants, workers contaminate products with pathogens such as *Staphylococcus aureus* during manual processing. A significant proportion of pathogens, particularly enteric viruses, are transmitted from produce to humans. Most fish parasites can cause disease while still in the larval stage. Fish parasites are transmitted to humans in various ways and have even been reported to cause death. Proper cooking and freezing methods prevent the transmission of pathogenic parasites to humans. The risk of spreading parasitic infections varies depending on the specific risks involved. We can list the disease-causing agents transmitted to humans through seafood that seriously harm human health as follows: bacterial agents, parasitic agents, and fungal agents [23].

Certain dishes prepared from fish, particularly those made with raw or undercooked fish, can pose certain risks to human health. Dishes such as sushi, sashimi, and carpaccio, which have become popular worldwide in recent years, are primarily made from raw fish; consequently, microbes, parasites, or harmful bacteria present in the fish can be directly transmitted to humans. Living in the intestine, these parasites can cause issues such as abdominal

pain, nausea, loss of appetite, weight loss, and allergic reactions. In some cases, these parasites can circulate through the body, leading to serious problems and sometimes even damage to internal organs. For example, microorganisms known by names such as *Aeromonas*, *Vibrio*, and *Salmonella* can cause gastrointestinal disorders, diarrhea, high fever, and sometimes even serious infections. These risks are particularly dangerous for children, the elderly, pregnant women, and individuals with weakened immune systems. These risks increase further when seafood products are not stored properly, sufficiently cleaned, or adequately cooked. Experts recommend that if raw fish is to be used, it must be frozen, purchased from a high-quality, certified source, and prepared in strict adherence to hygiene standards. Otherwise, certain infections transmitted by fish could pose a serious threat to public health. Therefore, it is important to approach the consumption of sushi and other raw fish dishes with caution and to be well-informed about the matter [24].

In fish farming, preventive measures are taken to prevent the occurrence of fish diseases. The implementation of preventive measures begins at the stage of farm design. Disinfectants and vaccination methods are primarily used during preventive measures. Disinfectants used for preventive purposes are considered highly effective substances for preventing disease outbreaks on farms, while being virtually harmless to fish. Disinfection in fish farms is primarily carried out using substances such as table salt, potassium permanganate, chlorinated lime, iodophores (Baticon, Vanodine), sodium hypochlorite, sodium hydroxide, and formaldehyde. Formaldehyde (formalin) is considered the most widely used effective agent for preventive purposes on farms. Based on international experience and considering the carcinogenic effects of malachite green and methylene blue—substances classified as disinfectants, the use of such agents during fish farming is not recommended [25].

5. Conclusion

Fish belonging to the order Cypriniformes are ecologically and economically significant species in the Caspian basin and associated water bodies. The biological diversity of the species included in this group and their large-scale cultivation in aquaculture simultaneously increase the risk of the spread of various infectious and parasitic diseases. Research results indicate that the most common diseases in cyprinids are of bacterial (aeromonosis, pseudomonosis, mycobacteriosis), viral (koi herpesvirus – KHV, fishpox), parasitic (ichthyophthiriosis, trichodinosis, myxosomatosis, diphyllbothriasis, anisakiasis), and fungal (saprolegniasis, ichthyosporidiosis, branchiomycosis) origin. The emergence of these diseases is closely linked primarily to deteriorating water quality, unsatisfactory zoohygienic conditions, unbalanced nutrition, stress factors, and weak immune response.

Anthropogenic changes in the environment, particularly pollution, the disruption of thermal regimes, and oxygen depletion, create conditions conducive to the activation of pathogenic microorganisms. In addition, intensive fishing activities and uncontrolled fish transfers play a significant role among the factors contributing to the epizootic spread of diseases. A comprehensive approach is required for control measures. Key methods include the use of antibacterial and antiparasitic drugs, disinfection of the aquatic environment, isolation of sick fish, and regular veterinary monitoring. However, these approaches are sometimes insufficient and result in pathogens developing drug resistance. However, these approaches are sometimes insufficient and result in pathogens developing drug resistance.

There are certain proposals listed below:

- The application of molecular and immunological methods for the diagnosis of diseases observed in catfish-like fish must be expanded.
- Biosecurity protocols must be developed for fish farms, and their implementation must be monitored.
- Veterinary inspection must be mandatory before fish products are placed on the market.
- An epizootiological monitoring system must be established at local and regional levels, and data on diseases must be collected and analyzed in real time.
- Strict control and awareness-raising measures must be implemented to preserve ecological balance, particularly to protect water bodies from anthropogenic pollution.

- Continuous training and professional development programs should be organized for fish farm owners and employees.
- Research activities and scientific collaborations should be expanded, and the exchange of information and technology with local universities and international institutes should be strengthened.

Ultimately, effective control of diseases affecting cyprinids is possible only through a comprehensive approach, scientifically grounded management, and preventive measures. This is also a crucial prerequisite for the sustainable development of the aquaculture sector and for supplying the population with healthy and safe fish products.

Author Contributions

The author conceived and designed the study, conducted the research, analyzed the data, and wrote the manuscript.

Conflict of Interest

The author(s) declare no conflicts of interest.

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