Available online at www.garmian.edu.krd

Passer Journal

Passer 7 (Issue 1) (2025) 1-16

http://passer.garmian.edu.krd/

Seasonal Impacts of Physical and Chemical Factors on Aquatic Fungi and Common Carp

Makwan Saeed Qadir*, Trifa Kamal Jalal, Sulaf Mustafa Mohammed

Department of Biology, College of Science, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq

Received 08 July 2024; revised 28 September 2024;
accepted 01 November 2024; available online 8 December 2024

DOI: 10.24271/PSR.2024.466813.1658

ABSTRACT

Saprolegniosis is recognized as one of the most widespread fungal diseases in freshwater fish, affecting fish and eggs of all ages and causing significant economic losses globally. The primary infectious agents are aquatic mold fungi belonging to the *Saprolegnia* spp. This study aimed to investigate the impact of physical and chemical factors on aquatic fungi and their relationship with common carp. This study was conducted over 12 months from July 1; 2021, to 1 July 2022, at Khurmal Pond, located in Sulaimani Governorate. Eight sites were selected, and a baiting method was used to isolate the aquatic fungus from water samples. The water temperature ranged between (11.3-26.6 centigrade °C), the pH ranges from 7.2 to 7.8 indicating a neutral to slightly essential, dissolved oxygen (DO) levels varied between 6 to 8.4 mg/L at the months of the experiment**,** total alkalinity (218-272 mg/L), the concentration of nutrient like as NO_3 (5.33-9.26 mg/L), SO_4 (30.3-35.3 mg/L) and PO_4 (0.15-0.64 mg/L). Twenty-five species of fungi were isolated in this detection: 17 species of Zoosporic fungi and eight species of conidial fungi representing eleven genera, namely: Zoosporic Fungi belonging to 5 genera *Achly*, *Aphanomyces*, *Dictyuchus*, *Pythium,* and *Saprolegnia,* Conidial fungi were belonging to 6 genera *Alternarea*, *Aspergillus*, *Curvulari*, *Fusarium*, *Pencillum* and *Rhizopous*. In conclusion, Saprolegniosis threatens freshwater aquaculture globally and explores how physical and chemical factors affect the occurrence and frequency of aquatic fungus in common carp disease.

Keywords: Fungus, Saprolegnia, Carp, Physical, Chemical, Factors.

1 Introduction

The steadily rising world population has resulted in a growing demand for protein, which has led to increased interest in fish as a source of high-quality protein. Fish farming plays a crucial role in meeting a significant portion of the demand for fish meat. Data from the FAO in 2020 indicate that global aquatic farming production has reached approximately 178.5 million tons [1]. Only a few fungal species are recognized as fish pathogens in fungal diseases. These organisms are mainly found in water, and they tend to exploit unfavorable circumstances to target fish, leading to skin ulcers. Most fungal infections documented in common carp farming are those caused by species associated with the *oomycete*, *Achlya*, *Aphanomyces*, and *Saprolegnia*. Infections caused by these fungi are collectively called *saprolegniasis* [2]. *Saprolegniasis* is a severe disease in the aquaculture industry that leads to economic losses and massive fish mortality, especially in winter [3]. It is known that *Saprolegnia* comprises about 14 genera, with 126 to 146 species, of which S. parasitica and *S. invaderis* are the most abundant. The fungal disease is common in freshwater with aquatic fish populations [4]. Both aquaculture industries and fisheries have gained more recognition in recent years for their important role in ensuring global nutrition and food security [5]. [6]**,** who described *S. parasitica* as one of the essential fungal infections contributing to economic loss in cultured ecosystems. An important goal of research in fish culture is to enhance cost-effective aquaculture production while providing adequate fish and animal protein by-products for human consumption. Saprolegniosis manifests as a cotton-like lesion on the head or dorsal fin that spreads to cover the entire body over time, eventually turning brown, red, or green. In untreated cases, Saprolegnia infection can result in death due to the failure of osmoregulatory function.[7]. *Saprolegniasis* in aquaculture is often associated with poor water quality and ecological stressors [8]. Oomycetes represent typical saprophytic opportunists, affecting stressed or injured fish; however, they have been reported as primary infection agents in fish [9]. The disease is caused by water molds (oomycetes), mostly in the genera Saprolegnia and Achyla [10]. *S. parasitica* is one of the primary agents of *saprolegniasis*, which can cause massive mortality in both fish and their eggs. *S. parasitica* is associated with losses in various fish species, such as the common carp, where the mold is responsible for more than 95% of cumulative mortalities under experimental conditions [11]. *S. parasitica*, which belongs to the order Saprolegniales [12]. Saprolegniales is one of the primary lineages of oomycetes. Saprolegniales have been traditionally known as water molds [13]. Fungal growth on the skin or fins manifests as patches resembling white to whitish-grey cotton-like

* Corresponding author
E-mail address makwanqadir@yahoo.com (Instructor).
Peer-reviewed under the responsibility of the University of Garmian.

growths. These formations primarily consist of numerous fungal hyphae, evident through microscopic analysis. The earliest aquaculture species for which historical records are available is the common carp (*Cyprinus carpio*). Since this species has been domesticated for a longer period than any other fish, it has given rise to a variety of variations, including koi fish, the partially scaled mirror carp *C. carpio var. specularis*, the orange-scaled scale carp *C. carpio var. flavipinnis* and the scale *fewer leather* carp *C. carpio var. nudus*. Eastern Europe and Asia are home to the omnivorous common carp, which eats food originating from plants, animals, and residue [14]. Any injuries, sudden ecological changes, poor water quality, and aquatic pollution are the primary predisposing factors of *saprolegniasis* in fish yield [15]. Infections initiate with the attachment of movable zoospores to injured areas. The hyphae consist of zoospores, which are deeply invasive to the lower tissues, leading to tissue damage and excessive ulceration. The obvious skin lesions differ depending on the species of the fish. Slightly infected fish exhibit mild inflammation without visible external wounds, but may also show petechial hemorrhages on the body, mouth, anal fin, and exophthalmos [16]. Ulcers initially appear as white spots on the fish's skin, which later change to red with a reddish center, eventually leading to external complications. As the disease progresses, symptoms include protruding eyes, body decay, and, in some cases, head erosion, which can lead to the death of the fish, depending on the severity of the disease [17]. These characteristics can change depending on many factors, including water quality [18]. Water quality and diet are supplementary, interdependent aspects. Any injury can decrease a fish's physiological and immune health, increasing its susceptibility to infection [19]. Fungi are eukaryotes that need oxygen for respiration. However, the oxygen level in water is much lower than in air, and as expected, fungal growth is greater in water bodies with higher dissolved oxygen levels. Further, fungi are known for their ability to degrade complex carbohydrates like cellulose, hemicelluloses, etc. Hence, fungal diseases are commonly viewed in floodplain wetlands and border of reservoirs, that generally have a high density of submerged macrophytes with (DO). Low water depth and the presence of submerged macrophytes in floodplain wetlands and along the periphery of reservoirs favor the growth of fungi belonging to the oomycete group that infects fish, especially during winter [20]. Fungal contamination of fish can be attributed to poor sanitation, workers' hands, water supply, and contaminated feed, all of which influence the fish's health status [21]. Controlling *saprolegniasis* requires adequate water quality parameters and good management practices [11]. The study aimed to identify aquatic fungi and fish pathogens like Saprolegnia spp. collected from farmed pond during one year, especially from an outbreak of *saprolegniasis*. Furthermore, the study estimates the physical and chemical parameters and their relationship to the growth of fungi in fish from pond cultures.

2 Materials and methods

2.1 Description of study area

The research was conducted in Khurmal District, Kurdistan Region, Iraq, at coordinates N 35°10'48.2556" E 45°59'10.7376. All fish ponds are between Grdi Go Village and Khurmal District, near Kalkuni Village (Mohammed Faraj), with the Zalm River lying nearby (Figure 1: B). The river plays a vital role in the socio-economic development of the area's residents, supporting activities such as livestock farming, irrigation, and fisheries. The river is the primary source of water supply for domestic use and consumption. The Kalkuni Village contains several fish ponds where the common carp (*Cyprino carpio*) is intensively reared and along 2 km contains several ponds (each generally measuring100 x 80 x 1.5 m in length, width, and depth), (Figures 1: A), that were fed two time daily at 5% of body weight, areas consisted of eight locations (L): 1, 2, 3, 4, 5, 6, 7 and 8. The sources of locations 1, 2, 3, and 4 are from a source named Kany, 200 meters from the first fish pond, while the second source, for locations 5, 6, 7, and 8, is from the Zalm River (Table 1). The area has a continental climate characterized by moderately dry, long, snowy winters and moderately warm, long summers. The month with the highest temperatures in the study area is July, with an average temperature of +39.6°C, while the coldest is January, with an average temperature ranging from 19.7°C to -5°C.

Table 1: Sites, coordinates, and ponds measurements.

Sites	Coordinate North (N)	Measurements
Location 1	N 35°18'22.3344" - E 46°0'19.3644"	110m, 60, 1.5m
Location 2	N 35°18'27.0612" - E 46°0'15.6456"	200m, 50m 1.5m
Location 3	N 35°18'27.1584" - E 46°0'12.6972"	100m, 30m, 1.5m
Location 4	N 35°18'27.7776" - E 46°0'10.4796"	200m, 80m, 1.5m
Location 5	N 35°18'30.4956" - E 46°0'10.332"	200m, 200m 1.5m
Location 6	N 35°18'31.0464" - E 46°0'7.1208"	80m, 15m, 1.5m
Location 7	N 35°18'30.8088" - E 46°0'5.1696"	100m, 70m, 1.5m
Location 8	N 35°18'32.1588" - E 46°0'1.3356"	100m, 70m, 1.5m

2.2 Sample collection

2.2.1 Isolation of Fungi from Water Pond

The baiting method isolated aquatic fungi from water samples collected from each site. A quantity of (15-20 ml) of pond water was pumped into a sterile petri dish. After placing 5-7 sesame seeds per petri dish, the containers were preserved in a controlled environment at 20°C for 7 days. The growth of fungus mycelia was removed from the seeds. The, samples were introduced and kept at 20°C for a maximum of 5 days on Sabouraud Dextrose Agar (SDA), contained chloramphenicol (250 mg antibiotic/L sterile distilled water) to depress bacterial growth from the environmental samples accompanied by routine daily monitoring for any anticipated fungal growth [22].

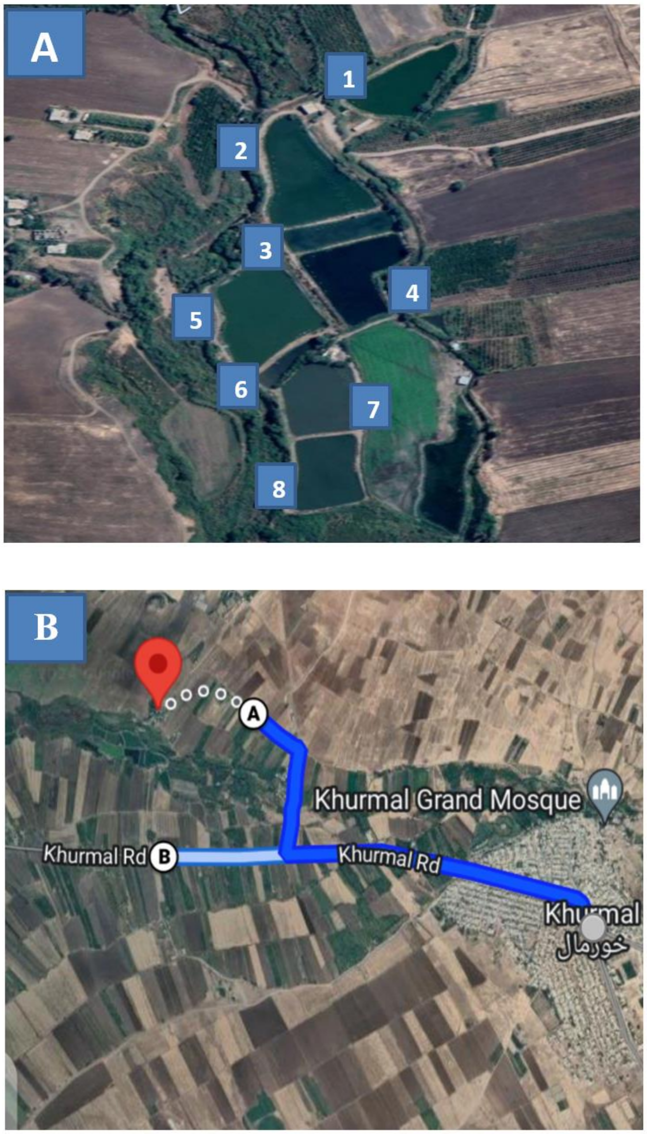

Figure 1: A- Satellite map showing the fish ponds (Satellite area). B- Showing distance from khurmal to Mohamad Faraj village 3.3 km.

After 5 days of being cultured on SDA, Corn Meal Agar (CMA), and Potato Dextrose Agar (PDA) media, according to Prasad *et al*. (2009), a whitish cottony growth emerged, along with long, hair-like structures [23]. To obtain a pure culture, sequential cultivation was performed by cutting a segment of growing mycelium and transferring it to a petri dish using a sterile surgical blade. The dish was then covered with a 2 mm layer of sterile tap water and incubated at 20°C for 5 days. The isolates were purified using the single hypha method. Identification of fungi was carried out with the help of various standard monographs [24], [25], [26].

2.2.2 Isolation of Fungi from Fish

1- Indirectly method: Different organs from common carp showing indications of fungal infection were carefully examined under a dissecting microscope and removed and placed onto SDA for injection [27], [28]. The inoculated sample was incubated at (20°C) for 5 days in the Department of Medical Laboratory Science (MLS) at the College of Health Sciences (CHS), University of Human Development (UHD). A small section of agar containing an emerging hyphal tip was carefully removed and transferred to a new medium for culture purification. The purified fungal forms were examined microscopically according to Shin et al., (2017) [29].
2- Direct Method: Skin lesions, infected muscles, and gills from the fish were swabbed and subsequently immersed in 0.1% sterile peptone water for the identification of aquatic fungi [30].

2.2.3 Selecting of aquatic fungi under microscopic

The morphology of the isolated culture was studied by preparing an oomycete sample on a slide and observing it using a BiOptic B-200 microscope equipped with an Industrial Digital Camera 3.0MP ½ COLOR USB2.0 APTINA CMOS SENSOR at magnifications of 120x and 600x. The fungal isolates were placed on slides with coverslips and then examined microscopically for spores and vegetative bodies. The fungi were identified based on colonial features and the micro-morphology of the spores they produced [31], [32].

2.3 Physico-chemical analysis of water samples

This study was conducted over 12 months, from 1 July 2021 to 1 July 2022, at a pond with eight sites. Eight water samples were collected each month from each site. The samples were gathered approximately 20 cm below the water surface at the experimental site for testing. Any water sample was collected in (250 ml) polyethylene plastic bottles. Samples intended for dissolved oxygen determination were collected in 250 ml glass Winkler bottles, adequately labeled, and taken to the laboratory to determine physical and chemical factors such as water temperature, pH, dissolved oxygen content, total alkalinity, nitrate (NO_3), sulfate ions (SO_4), and phosphate ions (PO_4). Water Temperature: The water temperature at each site was measured using a graduated mercury thermometer (0-100°C) during sampling. Ph: According to Fitri and Maulud (2020) [33], a pH meter (model H18915, Hanna, India) was used to measure the pH levels. Dissolved oxygen content: An oxygen meter measured the percentage of dissolved oxygen (DO), with results expressed in mg/L. The used German-made device is the 1350 multi-parameter sampler. Total alkalinity: Total alkalinity was determined using the titration method, and total dissolved solids were measured by evaporating a pre-filtered sample to dryness and calculating the mass of the dry residue per liter (mg/L) of a sample [34]. Nitrate (NO3) ion concentration was measured using the chromotropic acid [35]. Sulfate ion (SO4) concentration was estimated using the turbidity method, an optical technique with a spectrophotometer, as described by Wang *et al*. (2013) [36]. Phosphate ion: (PO_4) concentration was detected using the tin rose chloride method [33].

2.4 Statistical analysis

The analysis used GraphPad Prism (version 9.5.1; GraphPad Software Inc., San Diego, USA). Semiquantitative assessment scores of immunostainings were imported into the software. A two-way ANOVA was applied to evaluate physical and chemical water parameters across eight sites over 12 months, followed by Tukey's post-hoc test for multiple comparisons. All data are expressed as the mean ± standard error (SE), and p-values less than 0.05 were considered statistically significant [37].

3 Results and Discussion

3.1 Identification and isolation of aquatic fungous

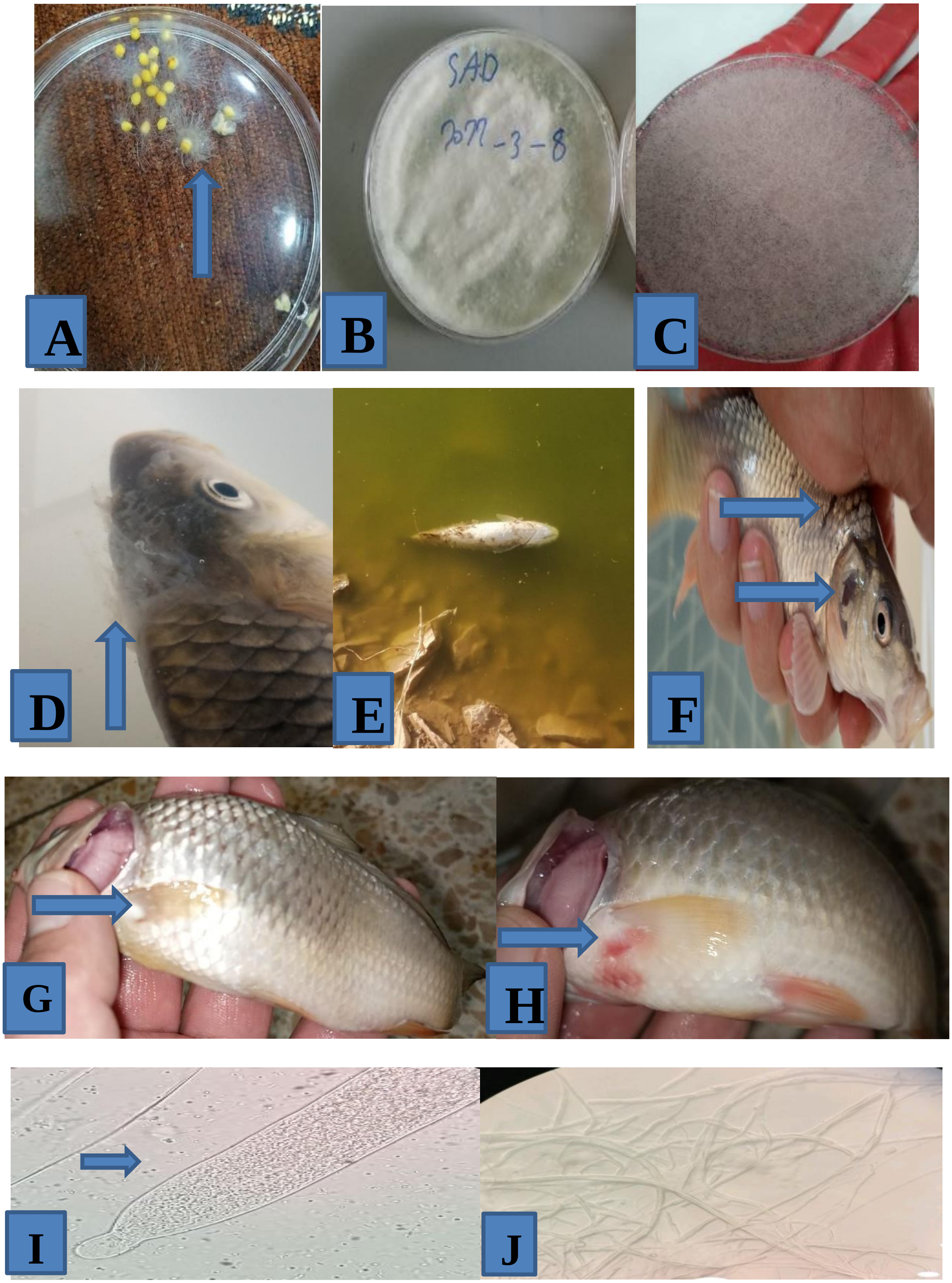

Figure 2: A, Daily inspections were performed to monitor the hyphal growth of aquatic fungus from the pond water samples, a wet culture of developing aquatic fungous on sesame seeds, *S. parasitica* can be grown on baits like sterilized (sesame seed) for the microscopic view of mycelia, zoospores and sexual reproductive structures [38]. B**,** growth of aquatic fungous from the Sabouraud Dextrose Agar (SAD) media after transferring smaller segment of hyphal growth from sesame seed, white cottony hairy root hyphal then isolate purified *Saprolegnia* on SDA or Saprolegnia species cultures started as long hairs with whitish cottony color. C**,** showing a fungal culture on SAD, which was isolated from fish infected with fungi D**,** Saprolegniosis, observed in numerous pond fish, is characterized by a white or greyish cotton-like tuft found primarily on the infected head, skin, and gills of affected fish. This disease has received less attention than many other significant diseases of freshwater fish, likely because fungal infections are often seen as opportunistic, primarily caused by mechanical injury during handling, exposure to extreme pH levels, prolonged exposure to low water temperatures, especially transitioning from winter to spring, inadequate food supply, and concurrent microbial infections [39]. E**,** death of common carp infected with *Saprolegnia parasitica*, observed in the pond**.** F**,** infections of common carp showing the manifestations of *Saprolegniosis*, white to grey patches on the outside body surfaces in both abounded gills and dorsal of carp fish. G, with grey to black lesions (arrows) of fungal infection on the body surface ventrally. H, cotton-wool-like masses on the ventral body surface, with hemorrhagic ulcers on the skin. Affected fish will be fragile reluctant to consume feed, and will swim at the surface. In the advanced stage of the disease, extensive fungal growth occurs with the appearance of a matted mass all over the body. The disease is relatively, pretty common in enclosure culture also, especially during winter months [8]. *Saprolegnia parasitica* employs multiple virulence factors, with its hyphae utilizing these factors against fish cells. In addition, Saprolegnia spores secrete adhesive materials capable of binding to lectins [40]. The transfer of effector proteins into infected cells facilitates the oomycete infection. *Saprolegnia parasitica* is a pathogen characterized by long-haired hook bundles rapidly generated during the secondary cyst phase. These bundles may serve as a potential pathogenic factor by increasing the ability of host epidermal cells to adhere to extracellular matrix proteins such as thrombospondin and fibronectin. This can lead to tissue damage and dehydration due to the release of bodily fluids and toxic substances [7]. I**,** microscopic examination of wet mount arrangement of Saprolegnia revealed characteristic aseptate hyphae, zoosporangia, and microscopic identification confirmed the presence of Saprolegnia spp. The zoosporangia in these individuals exhibit either a spherical shape, a cylindrical, or a significant quantity of spores. Assumption of a suspected Saprolegnia infection, detection of fungi from infected fish is carried out by cutting a small piece of infected gills, mussels, and skin about (2mm) in diameter from different parts of the body, thoroughly washed with sterilized distilled water to remove unwanted microorganisms adhered on the surface then growing them on selected media SDA as like Figure 2: B, C). J, the hyphae appeared profuse, separated, and non-septate when viewed under a microscope 400x. Fungal colonies are made up of complex, branched filaments known as hyphae. Some hyphae form a fungus network known as mycelium [4].

3.2 Isolated aquatic fungous

Twenty-six species of fungi were obtained in this investigation (17 species of zoosporic fungi and eight species of conidial fungi) representing 11 genera, namely: Zoosporic Fungi are *Achly*, *Aphanomyces*, *Dictyuchus*, *Pythium*, and *Saprolegnia*, were belonging to 5 genera five species of Achly, two species of *Aphanomyces*, two species of *Dictyuchus*, three species of *Pythium* and five species of *Saprolegnia*, Conidial fungi are *Alternarea*, *Aspergillus*, *Curvulari*, *Fusarium Penicillium* and *Rhizopus* were belonging to 7 genera: 1 species of *Alternaria*, three species of *Aspergillus*, one species of *Curvularia*, two species of *Fusarium*, one species of *Penicillium* and one species of *Rhizopus* (table 2). The higher total number of isolates acroos genra of *achlya* was *A. racemosa* included 47 species especially from winter there were 19 species, genra of *aphanomyces* is *stellatus* are 73 species especially from spring that has 21 species, genra of *dictyuchus* is *sterilis* are 32 species especially from autumn which has 10 species, genra of *pythium* is *ultimum* are 47 species in four seasons but the higher species of isolated in one season is spinosum that 14 species isolated in winter, genra of *Saprolegnia* is *parasitica* are 45 species especially from winter that has 17 species, totale isolated *Alternaria alternate* are 23 species especially from autumn and winter 7 species were isolated, genra of *Aspergillus* is *fumigatus* are 29 species especially from summer has 12 species (*Aspergillus fumigatus* grows best from 37 to 42 degrees C but can grow at temperatures up to 55 degrees C), total isolated species of *Curvularia lunata* are 4 species especially in summer that has 3 species, genra of *Fusarium* is *oxysorum* are 45 species especially from autumn that has 25 species, genra of *Penicillium* is *chrysogenum* are 14 species especially from summer has 7 species, genra of *Rhizopus* is *nigricans* are 5 species while 2 species were isolated from spring with summer. The highest total number of aquatic fungal isolates was 204, collected during the autumn season, while the highest total number of species was 25, isolated in both the autumn and spring seasons. The lower number of isolates and species observed in the summer, 143 isolates fungous and 22 species. This may be due to the high-water temperature (24 - 26.6 °C in summer), and high concentration of total sulfate in both (33.3 in summer, 35.3 in spring mg/I). Table 2: Seasonal variation shows that the richest season in aquatic fungal species frequency was autumn 2021 and winter 2022, Table 2: These seasonal represent low and moderate temperature; these results are under those obtained by [41]. Physico-chemical parameters of water samples used for the experiment indicated that the water temperature ranged from 13 - 26.6 °C. Water temperature variation was noted among the different sites due to environmental factors and topography. Water temperature was identified as the primary factor influencing the occurrence and frequency of aquatic fungal species. These results came in agreement with those investigated by [42]. The pH values ranged from 7.2 to 7.8 in the Kurdistan region of Iraq. Generally, the pH of the water shifted toward the alkaline side of neutrality due to the area's geology, which is primarily composed of $CaCO_3$ [43] similar conclusion observed by [44], [45]. The concentrations of nutrients such as nitrite, nitrate, and sulfate varied significantly across different seasons at all stations. These variations influenced the frequency and occurrence of aquatic fungi in both water and mud. *Achlya racemosa*, *Aphanomyces stellatus*, *Pythium spinosum*, *Pythium ultimum*, *Pythium angustatum*, *Saprolegnia parasitica*, *Fusarium oxysorum* and *Fusarium moniliforme* were the predominant species represented by 6.69%, 10.39%, 5.69%, 6.69%, 5.98%, 6.41%, 6.41% and 6.26% respectively. Whereas *Achlya Carolina*, *Achlya flagellate*, *Curvularia lunata*, and *Rhizopus nigricans* were observed rarely at 0.71%, 0.85%, 0.56% and 0.71%. The data obtained from the analysis regarding the occurrence and frequency of fungi in all the eight stations in four seasons, table 2: revealed the maximum percentage of fungi was recorded as *Aphanomyces stellatus* from four seasons of summer, autumn, winter, and spring (19, 17, 16 and 21 isolates), and *Saprolegnia parasitica* (1, 12, 17 and 15) from water samples respectively. This may be due to the characterization, low or moderate temperature (13 °C in autumn) and (11.1 - °C in winter) for water and slightly low concentration of nutrients (5.6 mg/I in autumn - 7 mg/I in winter nitrate), (0.15 in autumn - 0.23 in mg/I phosphate) this result is similar to Dudgeon *et al*., (2006) [46]. The main factories that have the highest number of fungal species in this research are located at the 5, 6, 7, and 8 pond from the Zalm River. This cause, an increase of different types of fungi as like [47]**,** Showing that in pond culture or any other culture environment, fishes in inland open waters are susceptible to parasites, fungi, bacteria, and viruses. Different fish species may vary in their susceptibility to various pathogens, and farmers and farm managers must be aware of the important diseases affecting the fish species stocked in and present in the waters of their interest. In addition to cultured species, wild fish in the water body can transmit pathogens to cultured fish. Bacterial fish pathogens are commonly present in water but may cause disease when their populations increase due to pollution. Disease outbreaks can also occur when fish are stressed or their immunity is compromised, particularly during winter [48].

3.3 Water quality

A commercial aquaculture enterprise's success depends on providing an ideal environment for the rapid development of aquatic organisms (fishes, prawns, etc.) at the lowest possible cost of resources and capital. The sum of all the biological, physical, and chemical parameters that impact the growth and welfare of cultured organisms is referred to as water quality. Water quality influences the overall state of cultured organisms since it directly or indirectly regulates their health and growth circumstances. Water quality is thus an important aspect to consider when planning for high aquaculture production. Human activities near water sources have a significant impact on water quality because water ecosystems and land are interconnected through surface runoff, groundwater systems and stream networks [49]. Deforestation, along with factors such as fish yield near water resources, could adversely affect water quality by increasing sedimentation and adding nutrients to water bodies [50]. Previously vegetated areas are increasingly being covered by built-up areas, and the intensification of land use continues degrading water quality [51].

3.4 Physical and chemical parameters in 12 months

3.4.1 Water Temperature

Temperature °C, tolerance limitations are well recognized in every type of organism, including fish. Temperature also alters the dissolved oxygen content in the water. Water temperature has a significant impact on fish ingestion variation, reproductive development, and breeding period. The chemical changes in both soil and water are greatly influenced by temperature. Temperature also plays a crucial role in stimulating gonadal development and spawning activity in carp [52]. In his findings, it was estimated that the optimal water temperature for the growth of Cyprinus carpio spawn is 26°C [53]. The research report shows that the best growth of common carp was obtained at a water temperature of 23–30°C. According to Table 3: and Figure 3, the water temperature of all sites has a more significant change between four seasons from one location to another, especially between summer with winter, autumn, and spring; this is evidence that all sites have the best condition for the growth of carp according to [54] who described that *Cyprinus carpio* could influence a wide range of temperatures from 3-35 °C range. The onset of Saprolegniosis is heavily impacted by temperature; most deaths attributed to Saprolegnia occur during late autumn, winter, and early spring; when outbreaks commonly arise in colder water temperatures [21]. Additionally, other stress factors, such as handling, poor water quality, and overcrowding, often coincide with outbreaks of Saprolegniosis [55]. Saprolegniosis predominantly occurs during cold temperatures and is commonly called "winter kill "[56]. [57] It was found that rapid reduction in water temperature affected the fish's immune system and temporarily suppressed mucus production by goblet cells in the skin's dermal layers. This mucus usually acts as a physical barrier, preventing fungal spores from attachment the fish's skin. Additionally, mucus contains antimicrobial elements such as proteolysis enzymes, lysozyme, C-reactive protein, and immunoglobulin, which can eliminate invasive zoospores. Without sufficient mucus, the skin becomes vulnerable, allowing fungi to increase and extend their hyphae into the muscle tissue.

Table 2: Isolation of fungal species from different locations during June 2021 to June 2022.

Name of fungous		Summer	Autumn	Winter	Spring	Total Number of Isolates	Frequency %
Zoosporic Fungi							
Achly spp							
Achlya apiculata de Bary		4	6	4	2	16	2.27
Achlya carolina Coker		0	2	2	1	5	0.71
Achlya racemosa Hildebrand		0	16	19	17	47	6.69
Achlya dubia Coker		3	3	5	6	17	2.42
Achlya flagellata Coker		0	2	3	1	6	0.85
Aphanomyces							
Aphanomyces laevis de Bary		1	5	6	2	14	1.99
Aphanomyces stellatus de Bary		19	17	16	21	73	10.39
Dictyuchus							
Dictyuchus sterilis Coker		6	10	9	7	32	4.55
Dictyocaulus magnus Coker		4	7	6	5	22	3.13
Pythium							
Pythium spinosum Sawada		9	9	14	8	40	5.69
Pythium ultimum Trow		11	17	9	10	47	6.69
Pythium angustatum Sparrow		8	10	11	13	42	5.98
Saprolegnia							
Saprolegnia australis Elliott		4	0	7	2	13	1.85
Saprolegnia ferax (Gruith)		7	9	6	8	30	4.27
Saprolegnia parasitica Coker		1	12	17	15	45	6.41
Saprolegnia diclina Humphrey		1	6	9	7	23	3.27
Saprolegnia delica Coker		0	5	8	5	18	2.56
Conidial fungi							
Alternarea							
Alternaria alternata (fr kreisisier)		5	7	7	4	23	3.27
Aspergillus							
Aspergillus niger Van Tieghem		9	3	10	4	26	3.70
Aspergillus fumigatus Fresenius		12	3	9	5	29	4.13
Aspergillus flavus Antonio Micheli,		9	3	7	3	22	3.13
Curvularia lunata		3	1	0	0	4	0.56
Fusarium							
Fusarium oxysorum Schlech tendal		6	25	5	9	45	6.41
Fusarium moniliforme		7	23	6	8	44	6.26
Pencillum							
Penicillium chrysogenum Thorn		7	3	3	1	14	1.99
Rhizopous							
Rhizopus nigricans		2	1	0	2	5	0.71
Total Number Of Isolates		143	204	190	166	702	
Total Number Of Species		22	25	24	25		

Table 3: Average water temperatures taken from different locations in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	24.6 ± 1.13	13.0 ± 0.96	12.0 ± 1.33	20.0 ± 1.45
L2	23.0 ± 0.85	13.0 ± 0.49	11.3 ± 1.20	19.6 ± 2.33
L3	26.6 ± 1.48	13.0 ± 1.36	12.6 ± 0.57	19.3 ± 0.66
L4	25.0 ± 1.85	13.3 ± 1.66	11.6 ± 0.33	18.3 ± 0.66
L5	26.3 ± 1.96	13.9 ± 2.17	12.6 ± 0.33	18.4 ± 1.59
L6	25.0 ± 0.55	13.1 ± 1.30	11.7 ± 0.85	18.8 ± 0.41
L7	26.0 ± 1.48	13.1 ± 1.29	13.1 ± 0.59	18.6 ± 1.42
L8	24.3 ± 0.59	13.0 ± 1.16	11.3 ± 0.57	19.6 ± 0.61

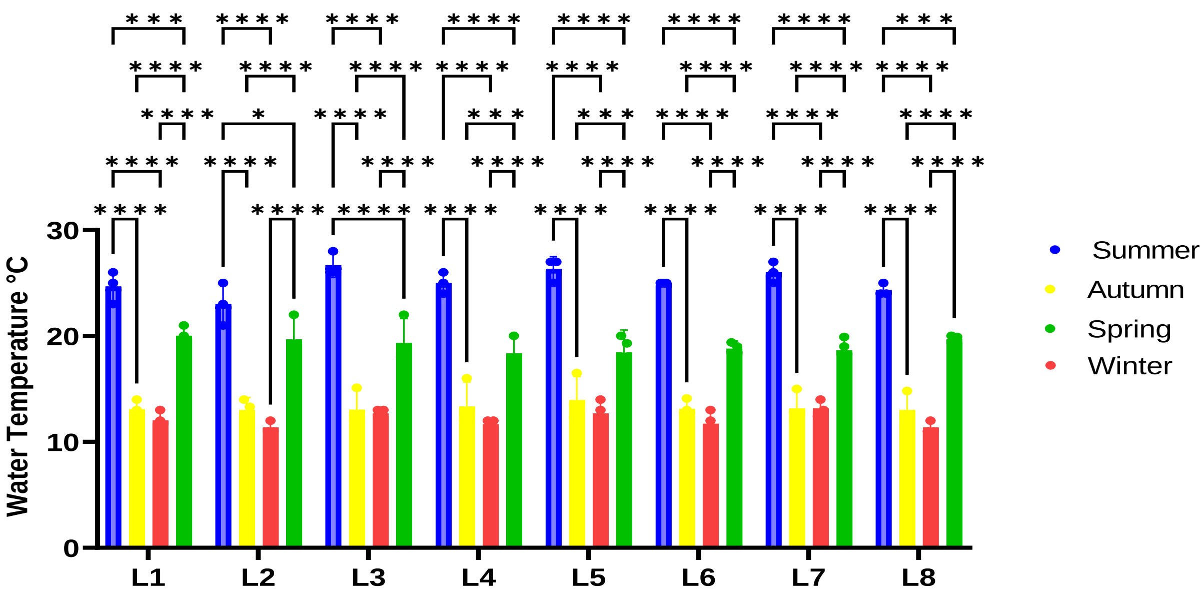

Figure 3: View the vital difference in the water temperatures recorded in seasonal change between different sites.

3.4.2 pH

The concentration of hydrogen ions in water is measured as pH, which shows the acidity and alkalinity (basicity) of the water. Fish metabolic and physiological processes are affected by Ph. The optimum pH required for the growth of carps is 7.5 to 8.3 [58], Sapkale *et al*., (2011) [52]. That the, estimated best pH is 7.3 and can promote maximum growth of *Cyprinus carpio* spawn. According to HEYDARNEJAD (2012) [59], common carp survive and thrive best when exposed to water pH levels ranging from 7.5 to 8.0. During the recent study, the pH ranged in Table 4: The pH values ranged from 7.2 to 7.8, with slight variations observed during the sampling months from autumn to spring. Iraqi waters are generally alkaline. Several factors, such as the breakdown of algae and organic matter in aquatic plants, influence hydrogen ion levels in aquatic environments. Photosynthesis by these organisms consumes CO2, while respiration releases CO2, creating a balance that can lower pH values in the water column [60]. According to Raes *et al*., (2009) [61] the common carp can live within a pH range of 6.5 to 9. Table 4 and Figure 4 show no significant changes between the different sites.

Table 4: Pond water pH according to seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	7.5 ± 0.36	7.3 ± 0.10	7.3 ± 0.32	7.8 ± 0.17
L2	7.5 ± 0.26	7.3 ± 0.12	7.3 ± 0.21	7.6 ± 0.41
L3	7.4 ± 0.22	7.3 ± 0.19	7.2 ± 0.16	7.6 ± 0.28
L4	7.4 ± 0.16	7.5 ± 0.11	7.4 ± 0.20	7.7 ± 0.45
L5	7.4 ± 0.49	7.7 ± 0.22	7.7 ± 0.27	7.4 ± 0.14
L6	7.6 ± 0.26	7.3 ± 0.17	7.4 ± 0.23	7.5 ± 0.35
L7	7.6 ± 0.20	7.3 ± 0.24	7.4 ± 0.40	7.2 ± 0.32
L8	7.4 ± 0.29	7.3 ± 0.02	7.3 ± 0.28	7.5 ± 0.26

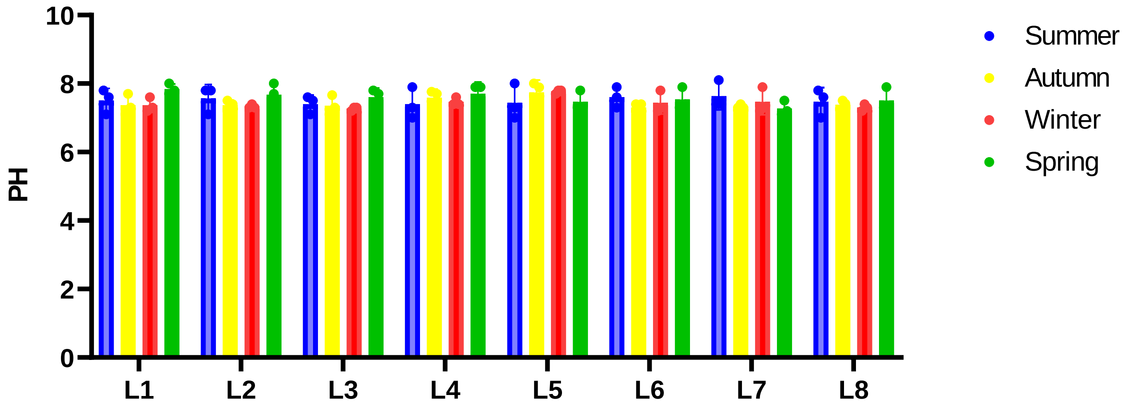

Figure 4: Level of pH in the different locations during the time.

3.4.3 Dissolved Oxygen

Dissolved oxygen (DO) is one of the vital factors that impair the growth of biological entities in the aquatic environment [62]. The most, essential factor in preserving fish life and survival is dissolved oxygen. The optimal DO concentration for a fish pond is customarily maintained at five mg/L to the saturation level for healthy fish growth and reproduction in tropical waters. During the present study, dissolved oxygen levels ranged between 6 and 8.4 (mg/L). According to Bhatnagar and Singh 2010) [63], a DO level of more than five ppm is required for excellent fish production. The toxicity of heavy metals increases when dissolved oxygen levels are low, potentially damaging fish gills and necessitating increased respiration for optimal fish health. According to the results presented in Table 5 of the current study, dissolved oxygen levels were higher at 8.4 mg/L during winter and lower at six mg/L in summer. This finding contrasts with expectations. The elevated dissolved oxygen levels in March are likely because of the growth of aquatic plants and continuous water movement. Conversely the decline in DO in October and November may result from reduced aquatic plant presence. Dissolved oxygen is a crucial metric in fish farming because it represents the chemical, physical, and biological processes in the water. Throughout the year, its concentration is five mg/L, and the DO value reflects the degree of pollution in water bodies [64]. The recorded levels of ecological factors during the study period are suitable for the growth of common carp, as indicated by Salman (2000) [65], who stated the lower level of DO, in water for Cyprinidae should not be less than (3 mg/L), [66] also showed the capability of rearing common carp fingerlings in coastal waters with salinity surpassing more than six ppt with a survival rate attained 100%. As shown in Table 5 and Figure 5, it is clear that there is no change in the rates of dissolved oxygen concentrations across all sites of pond water examined during the study period.

Table 5: General rates of (DO) concentration in the eight-pond water during the study in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	6.7 ± 0.96	7.6 ± 0.57	8.1 ± 1.51	8.3 ± 1.42
L2	6.9 ± 1.80	7.7 ± 0.15	7.8 ± 1.64	7.8 ± 1.73
L3	7.5 ± 0.31	7.5 ± 0.13	7.4 ± 0.44	7.3 ± 0.17
L4	7.1 ± 0.40	7.6 ± 0.64	8.4 ± 0.63	8.4 ± 0.29
L5	7.3 ± 0.23	7.7 ± 0.28	7.9 ± 0.45	7.8 ± 0.37
L6	6.8 ± 0.40	7.8 ± 0.12	7.7 ± 0.49	8.0 ± 0.37
L7	6.0 ± 0.22	7.6 ± 0.33	7.4 ± 0.40	7.7 ± 0.52
L8	6.8 ± 0.85	7.3 ± 0.20	7.6 ± 0.86	7.9 ± 0.80

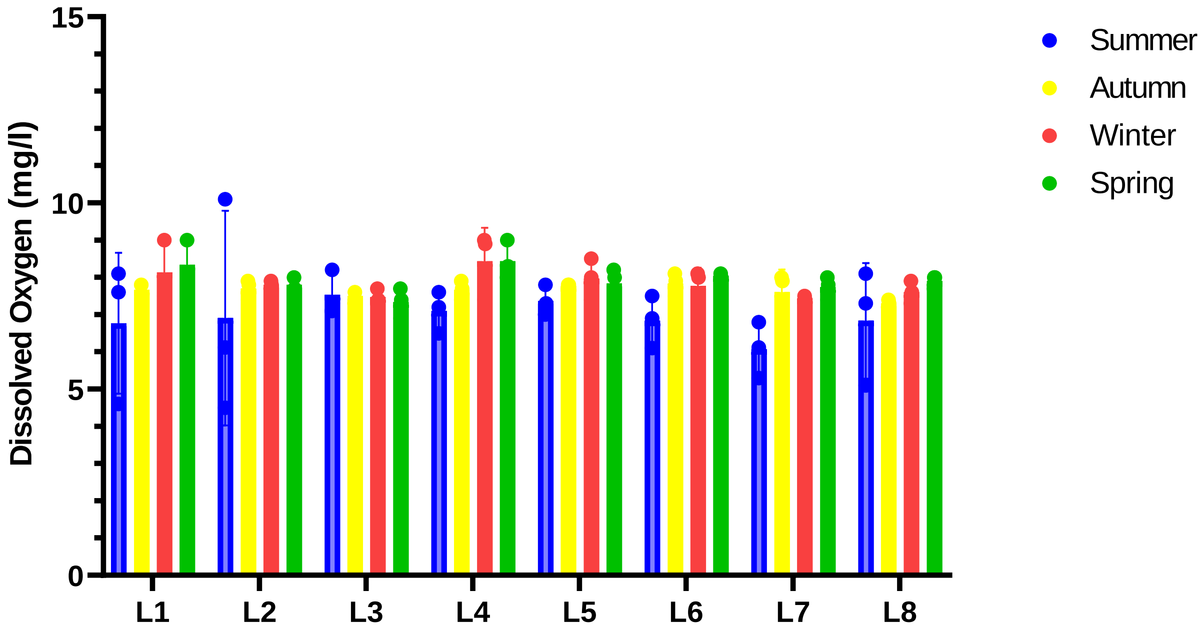

Figure 5: The concentration of (DO), in the different pond sites with the significant change during the study.

3.4.4 Total Alkalinity

Alkalinity refers to the total amount of bases in water. Ponds with an alkalinity of 50 ppm are considered productive, while ponds with an alkalinity of 90–100 ppm are considered highly productive. According to Bauer (2023) [58]**,** during the recent study from Table 6, the alkalinity range was found to be between 260 in summer and 218 in winter (mg/L). This is, the opposite; a total alkalinity of 80-120 (mg/L) is ideal for carp's growth. According to Alikunhi (1957) [67], the alkalinity in highly productive water should be greater than 100 ppm. But our result was approved by Parida et al., (2007) [68], which is clear that 50-300 (mg/L) alkalinity is optimum for fish production. Table 6 and Figure 6 show no significant changes between the different sites, except at location 8, where significant changes were observed between summer and winter, as well as between winter and spring.

Table 6: Shows the value of alkalinity in the ponds during the study in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	242 ± 17.7	252 ± 18.5	218 ± 8.74	226 ± 4.91
L2	255 ± 10.8	231 ± 23.3	244 ± 34.0	260 ± 19.8
L3	258 ± 15.9	256 ± 13.1	237 ± 3.48	272 ± 13.3
L4	236 ± 7.21	237 ± 8.73	230 ± 4.72	239 ± 1.76
L5	261 ± 28.4	232 ± 16.1	231 ± 13.0	244 ± 12.5
L6	256 ± 25.7	248 ± 18.4	248 ± 8.35	245 ± 8.41
L7	243 ± 7.21	244 ± 16.5	240 ± 10.0	237 ± 13.3
L8	265 ± 16.7	241 ± 1.45	220 ± 15.3	271 ± 11.7

3.4.5 NO_3

According to the outcome of the present study, nitrate levels increased in spring, summer, and autumn, especially in spring, reaching 9 (mg/L). High temperatures, which stimulate the activity of nitrifying bacteria, or rainfall, may be responsible for the increase in nitrates [69]. Nitrate is created when aerobic bacteria convert NO_2 to NO_3 through nitrification. If it is not promptly assimilated by aquatic plants, it is denitrified in anaerobic sediments [70]. Table 7: shows the lower concentration of nitrate is 5.333 (mg/L) in summer; because the source of all eight-pond water is groundwater, the levels of NO_3 from summer are lower, as evidenced by this research; nitrate levels in groundwater are typically low, although they can rise due to runoff or leaching from agricultural fields. The metabolic oxidation of ammonia results in the formation of nitrates. Due to adverse health impacts and the effects of nitrates on people and animals, it is crucial to monitor nitrate levels in drinking water supplies [71]. Research has reported that nitrate levels between 60 and 100 mg/L can cause deformities, abnormal swimming, and general health issues in rainbow trout and common carp [72]. Therefore, the NO_3 levels at all sites are considered safe for the health of common carp. In Table 7 and Figure 7, no change in NO_3 levels was observed between the different groups, except at Site 8, where a significant difference was found between summer and spring. The nitrate concentration in drinking water is from 0 to 32.2 mg/L. The average (NO_3) concentration in tap water was (8.96 ± 2.8 mg/L), which is below the accepted standard of 50 mg/L [73].

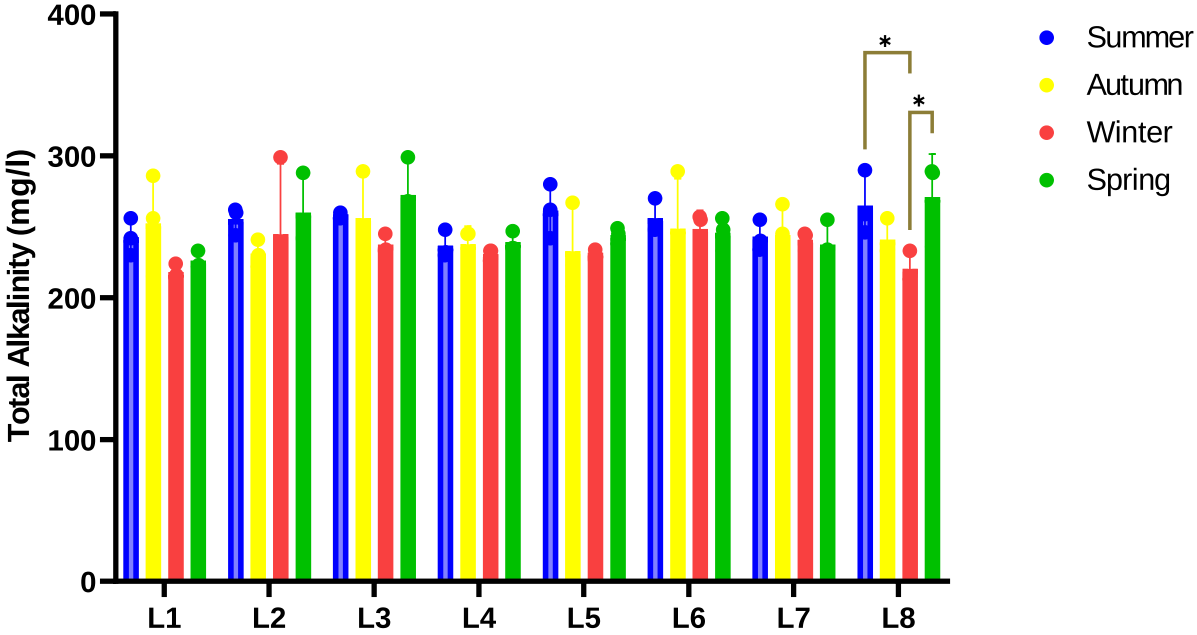

Figure 6: The alkalinity level in the different locations of fish ponds and the significant change between the sites in 12 months of the study.

Table 7: The average nitrate ion concentration (NO_3) across all pond sites during the study months in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	6.50 ± 0.76	7.00 ± 1.18	9.26 ± 1.65	9.00 ± 1.50
L2	7.53 ± 0.20	6.33 ± 0.33	8.00 ± 0.53	8.33 ± 1.33
L3	7.33 ± 0.37	7.00 ± 0.33	7.33 ± 0.68	8.33 ± 0.75
L4	9.10 ± 0.39	8.33 ± 1.20	7.66 ± 0.86	9.00 ± 0.10
L5	7.36 ± 1.28	9.60 ± 0.26	8.10 ± 1.15	8.66 ± 1.48
L6	7.83 ± 1.16	5.66 ± 0.39	7.90 ± 1.55	8.66 ± 1.09
L7	6.33 ± 2.08	6.33 ± 0.88	7.00 ± 1.20	8.66 ± 1.20
L8	5.33 ± 0.93	6.36 ± 1.75	7.00 ± 1.20	8.66 ± 0.33

3.4.6 SO_4

Sulfate ions are produced from various processes and sources, primarily the dissolution of gypsum and the reaction of sulfur dioxide gas with water. Additionally, the biodecomposition of organic materials high in sulfur contributes to sulfate ion production. Sulfate ions are among the most prevalent negative ions found in water [74]. The elevated concentration was observed due to the dissolution of sediments and gypsum rocks, which have disseminated and covered a significant portion of the spring's formation area [75]. Sulfate concentrations in natural water range from a few to several hundred mg/L, yet no significant adverse effects on human health have been noted. In Table 8, the concentration of sulfate varied from 30.33 to 34.67 (mg/L). The WHO drinking water standards set a limit of 250 mg/L [71], proving that water concentration at all locations does not affect human health. Based on the confirmed results of SO_4, the water from the studied stations may be deemed unsuitable for drinking due to its high sulfate ion concentration. However, it could potentially be used for industrial purposes or irrigation. Elevated sulfate ion levels impart a bitter taste to the water, with the intensity varying based on the dissolved sulfate level. When sulfate ion concentrations exceed 500 mg/L in groundwater, it produces a noticeable bitter taste [73]. In Table 8 and Figure 8, no changes in SO_4 levels were observed between the different groups, except at Site 5 and Site 8, where significant differences were noted: between autumn and spring at Site 5, and between winter and spring at Site 8.

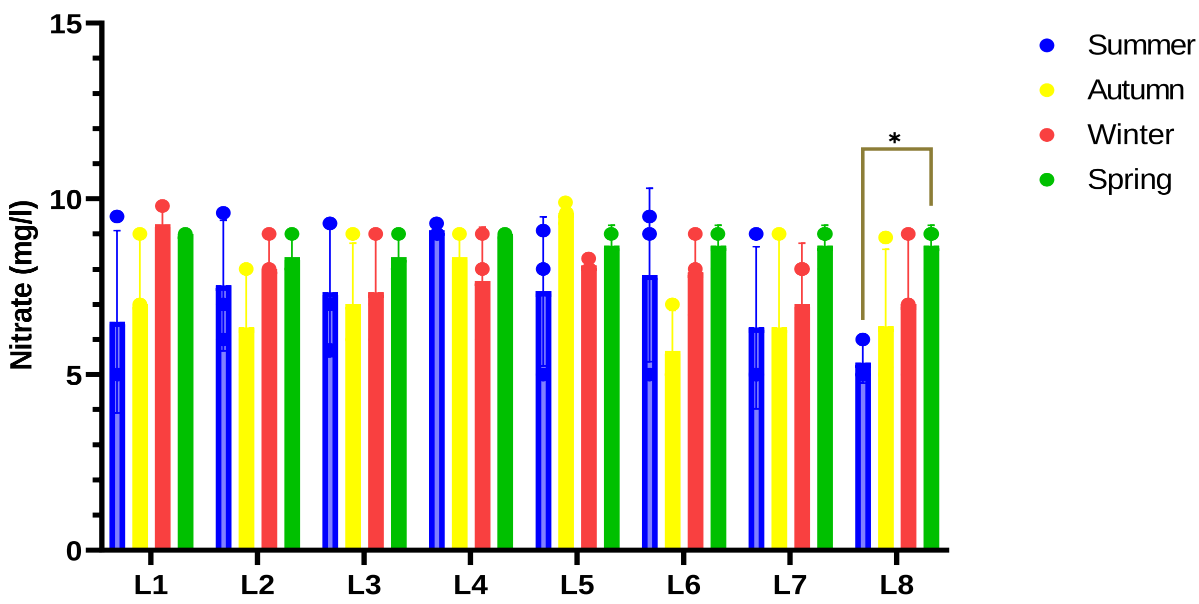

Figure 7: Demonstrates the overall levels of (NO_3) in water across various locations during the study period and significant differences observed across different months of the study.

Table 8: The average sulfate ion concentrations (SO_4) in the water across various locations during the study months in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	32.3 ± 1.33	32.0 ± 1.95	32.1 ± 1.33	34.6 ± 1.20
L2	32.3 ± 0.33	32.0 ± 2.18	32.3 ± 2.08	34.3 ± 1.15
L3	32.7 ± 0.29	32.3 ± 2.08	31.3 ± 1.94	33.3 ± 0.80
L4	32.0 ± 0.73	31.3 ± 0.66	30.6 ± 1.40	34.0 ± 2.94
L5	31.7 ± 0.86	31.3 ± 0.88	33.6 ± 1.56	35.3 ± 1.94
L6	32.4 ± 1.29	33.6 ± 1.00	32.6 ± 0.95	33.3 ± 1.62
L7	33.3 ± 1.45	32.6 ± 1.66	32.0 ± 0.66	34.3 ± 0.57
L8	32.6 ± 1.20	33.0 ± 2.18	30.3 ± 1.45	34.6 ± 1.15

3.4.7 PO_4

Phosphates are another element that can degrade water quality in natural resources. They are extensively used as agrochemicals in fish ponds and can infiltrate groundwater or surface waters through aquaculture, urban wastewater, and agriculture. While PO_4 substances are harmless to humans, they can diminish DO levels in water bodies [76]. Phosphorus pollution in watery ecosystems leads to severe problems, including harmful algal blooms, fish mortality due to reduced dissolved oxygen levels, biodiversity loss, and degradation of coral reefs and aquatic plant beds [77]. Excessive phosphorus in water causes acidification and lower oxygen levels, disrupting the marine food web and creating dead regions in the ecosystem. These ecological consequences pose risks to the tourism industry [78]. Phosphate is a critical factor influencing aquatic biological growth, such as phytoplankton and plants. In environments where it is less abundant, organisms rely on dissolved inorganic phosphate for their development [79]. Soluble Reactive Phosphorus (PO_4) is an essential nutrient and a deciding element for water reservoir fertility. It is also recognized as a significant factor produced by fish farms and influences the lake ecosystem [80]. The phosphate concentration in our current study reached 0.1- 0.6 (mg/L), near to what was discovered in the study [81] where, which is adequate according to Iraqi drinking water criteria [82]. The (US) Environmental Protection Agency has set a maximum allowable PO_4 content of (0.03) mg/L for tap water. PO_4 is an essential nutrient for lakes and rivers, vital for completing the natural food chain. However, excessive phosphate can lead to other secondary pollution issues and algal blooms. Fertilization practices in both agricultural areas and aquatic ponds can increase phosphate levels in water. Our study investigates the impacts of fish density and fertilization in aquaculture farms on phosphate and nitrogen pollution in nearby and distant water resources across different months of the year. In common carp ponds, fertilization with nitrogen fertilizers and animal waste like ammonium sulfate, nitrate, and phosphate is standard practice. This fertilization introduces significant nutrients into ponds, promoting the growth of phytoplankton. Our findings indicate that fertilization significantly raises levels of both nitrite and nitrate, with phosphate in aquaculture ponds, impacting water pH and dissolved oxygen (DO) fluctuations. We observed higher phosphate levels in summer compared to autumn, winter, and spring, with river phosphate concentrations exceeding those in aquaculture ponds and wells [83]. In Table 9 and Figure 9, no change in PO_4 levels was observed between the different groups, except at Site Eight, where a significant difference was observed between autumn and winter.

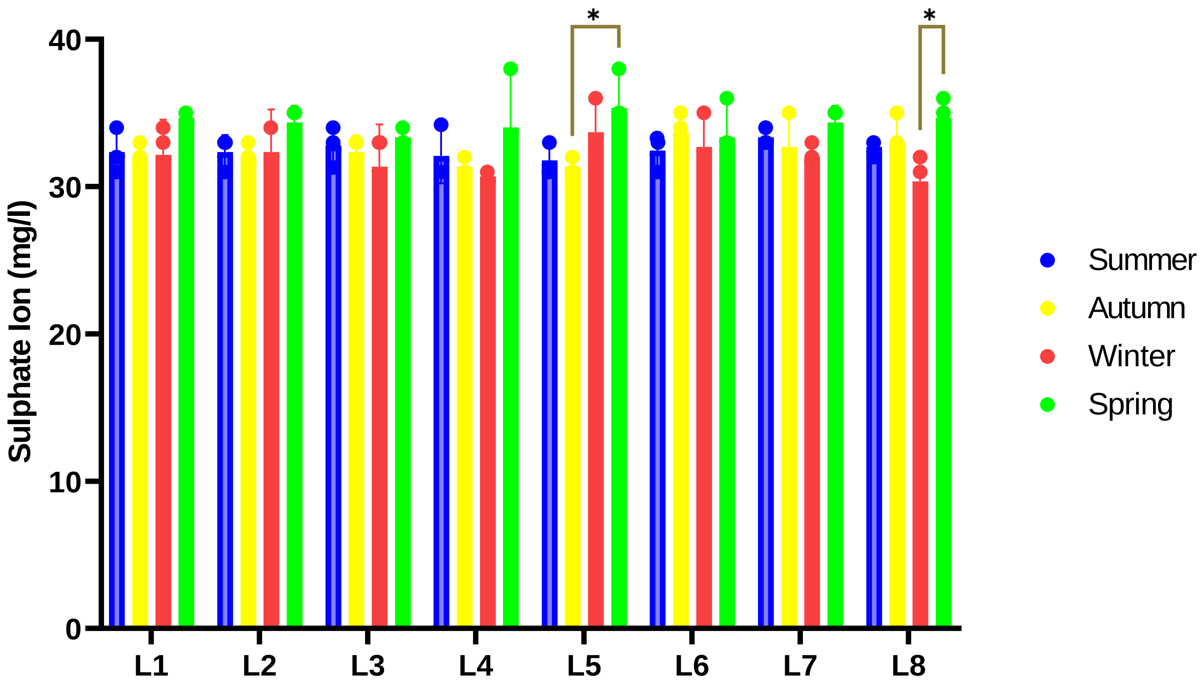

Figure 8: Demonstrates the overall average levels of SO_4 in the water across different locations during the study period, highlighting significant differences observed among the values across different months of the study.

Table 9: General rates of PO_4 in the water of the site during the study months from different locations in four seasons (Mean±SE).

Locations	Summer	Autumn	Winter	Spring
L1	0.53 ± 0.09	0.56 ± 0.10	0.35 ± 0.07	0.36 ± 0.06
L2	0.39 ± 0.09	0.43 ± 0.31	0.53 ± 0.30	0.36 ± 0.10
L3	0.46 ± 0.22	0.43 ± 0.01	0.50 ± 0.22	0.63 ± 0.21
L4	0.61 ± 0.09	0.46 ± 0.14	0.52 ± 0.11	0.43 ± 0.12
L5	0.42 ± 0.27	0.40 ± 0.04	0.23 ± 0.22	0.39 ± 0.13
L6	0.51 ± 0.18	0.64 ± 0.04	0.37 ± 0.14	0.40 ± 0.24
L7	0.40 ± 0.12	0.48 ± 0.04	0.40 ± 0.08	0.50 ± 0.08
L8	0.33 ± 0.15	0.15 ± 0.14	0.63 ± 0.04	0.43 ± 0.15

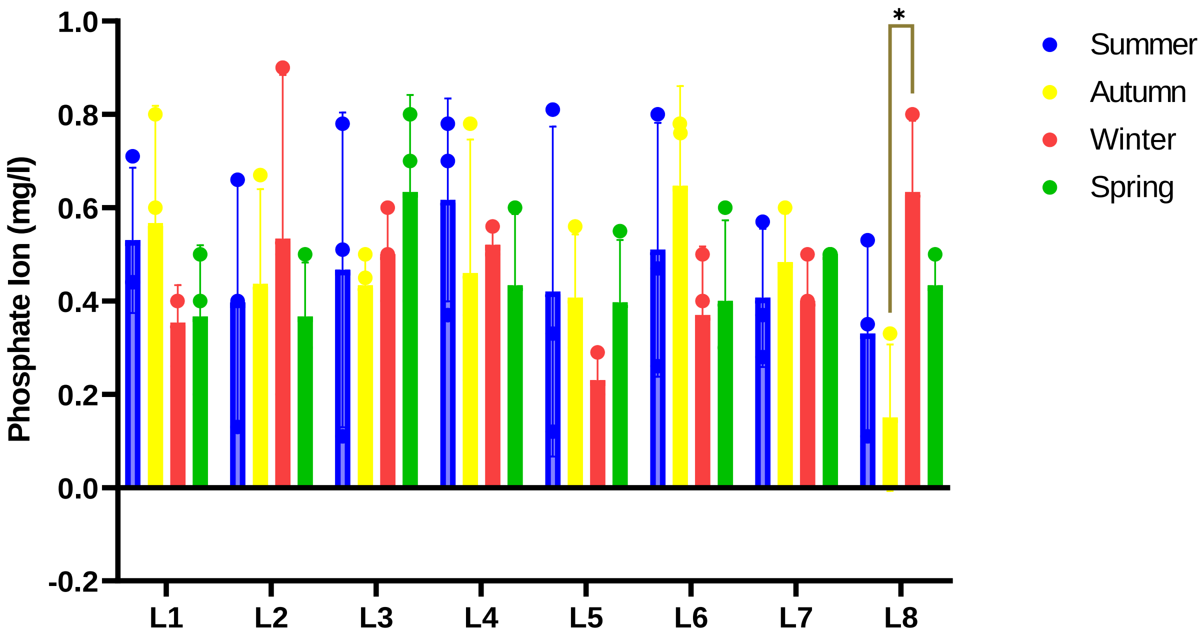

Figure 9: The levels of PO_4 in the water at different locations showed significant changes during the study period.

Conclusion

In this study, several genera of aquatic fungi and fungus-like organisms (Zoosporic Fungi are *Achly*, *Aphanomyces*, *Dictyuchus*, *Pythium* and *Saprolegnia* Conidial fungi are *Alternarea*, *Aspergillus*, *Curvulari*, *Fusarium Penicillium* and *Rhizopus*) were determined which they grew on the fish pond in kulkuni village (Mohamed Faraj), as well as the effect of the ecological factors, including particular physical and chemical analysis of water like as water temperature, Ph, total, dissolved oxygen, total alkalinity, NO_3, SO_4, and PO_4 were the optimum range and all the parameter effects on growth of fungus and growth of fish. Fungal infections play a significant role in fish diseases. There is a substantial amount of literature on fungal diseases in fish, and controlling these diseases is vital to ensure continued growth in the aquaculture production industry.

Ongoing research into the underlying molecular processes of fungal-host fish interactions is essential.

Conflict of interests

None.

Author's contribution

In a demonstration of the cooperative environment, each author contributed equally crucial ideas. The researchers meticulously devised and implemented the research framework, followed by a thorough data analysis and integration of their findings into a cohesive manuscript. Their seamless teamwork and combined expertise drove each phase of this effort, affirming this work as a true testament to our shared dedication.

Funding

The publication and authorship of this article followed an independent investigation that did not receive any external financial assistance.

References

[1] Canton, H. Food & Agriculture Organization of the United Nations—FAO. The Europa Directory of International Organizations **2021**, 297–305, Routledge (2021).

[2] Mishra, S., Dwivedi, P. D., Pandey, H. P. & Das, M. Role of oxidative stress in Deoxynivalenol induced toxicity. *Food Chem Toxicol* **72**, 20–29, doi:10.1016/j.fct.2014.06.027 (2014).

[3] Nam, K., Kim, J. & Lee, S. Genome-wide study on 72,298 individuals in Korean biobank data for 76 traits. Cell Genomics **2**, 100189, doi:10.1016/j.xgen.2022.100189 (2022).

[4] Yıldız, R., Ekici, H. & Yarsan, E. Antioxidant effects of Viburnum opulus on streptozotocin-induced experimental diabetic rats. *J Appl Biol Sci* **14**, 124–135, doi:10.1234/jabs.2020.14.124 (2020).

[5] Moura, R., Pessanha Santos, N., Vala, A., Mendes, L., Simões, P., de Castro Neto, M. & Lobo, V. Fisheries Inspection in Portuguese Waters from 2015 to 2023. *Sci* Data **11**, 362, doi:10.1234/sci.2024.11.362 (2024).

6] Zahran, S., Iverson, T., McElmurry, S.P. & Weiler, S. The effect of leaded aviation gasoline on blood lead in children. *J* of the Association of Environmental and Resource Economists **4**, 575–610 (2017).

[7] Srivastava, V., Rezinciuc, S. & Bulone, V. Quantitative proteomic analysis of four developmental stages of Saprolegnia parasitica. *Frontiers in Micro* **8**, 2658, doi:10.3389/fmicb.2017.02658 (2018).

[8] Ali, S.A., Baloch, M., Ahmed, N., Ali, A.A. & Iqbal, A. The outbreak of Coronavirus Disease 2019 (COVID-19)—An emerging global health threat. *J of Infection and Public Health* **13**, 644–646, doi:10.1016/j.jiph.2020.02.033 (2020).

[9] Roberts, C. Information structure: Towards an integrated formal theory of pragmatics. Semantics and Pragmatics **5**, 6–1, doi:10.3765/sp.5.6 (2012).

[10] Zhang, S. & Duan, E. Fighting against skin aging: the way from bench to bedside. Cell Transplantation **27**, 729–738, doi:10.1177/0963689717725755 (2018).

[11] Ali, H., Khan, E. & Ilahi, I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence,

toxicity, and bioaccumulation. *J of Chem* **2019**, 6730305, doi:10.1155/2019/6730305 (2019).

[12] Wu, W.Q., Wang, Q., Fang, Y., Shao, Y., Tang, S., Deng, Y., Lu, H., Liu, Y., Li, T., Yang, Z. & Gruverman, A. Molecular doping enabled scalable blading of efficient hole-transport-layer-free perovskite solar cells. Nature Communications **9**, 1–8 (2018).

[13] Svoboda, B.E., Shirley, Y.L., Traficante, A., Battersby, C., Fuller, G.A., Zhang, Q., Beuther, H., Peretto, N., Brogan, C. & Hunter, T. Alma observations of fragmentation, substructure, and protostars in high-mass starless clump candidates. *The Astrophysical J* **886**, 36, doi:10.3847/1538-4357/ab40ca (2019).

[14] Kültz, D., Gardell, A.M., DeTomaso, A., Stoney, G., Rinkevich, B., Rinkevich, Y., Qarri, A., Dong, W., Luu, B. & Lin, M. Deep quantitative proteomics of North American Pacific coast star tunicate (Botryllus schlosseri). Proteomics **2024**, 2300628, doi:10.1002/pmic.202300628 (2024).

[15] Roberge, P.R. Corrosion inspection and monitoring. John Wiley & Sons (2007).

[16] Iberahim, N.A., Trusch, F. & Van West, P. Aphanomyces invadans, the causal agent of epizootic ulcerative syndrome, is a global threat to wild and farmed fish. *Fungal Bio Reviews* **32**, 118–130, doi:10.1016/j.fbr.2018.05.002 (2018).

[17] Podeti, K.R. & Benarjee, G. Haematological changes in South Indian fresh water murrel, Channa punctatus, have both EUS and A. hydrophila infection. *J of Parasitic Dis* **41**, 329–335, doi:10.1007/s12639-016-0798-x (2017).

[18] Fazio, L.K., Kennedy, C.A. & Siegler, R.S. Improving children's knowledge of fraction magnitudes. *PloS One* **11**, e0165243, doi:10.1371/journal.pone.0165243 (2016).

[19] Imran, M., Das, K.R. & Naik, M.M. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. *Chemosphere* **215**, 846–857, doi:10.1016/j.chemosphere.2018.10.114 (2019).

[20] Lategan, M.J., Torpy, F.R. & Gibson, L.F. Control of saprolegniosis in the eel Anguilla australis Richardson, by Aeromonas media strain A199. *Aqua* **240**, 19–27, doi:10.1016/j.aquaculture.2004.04.009 (2004).

[21] Kumar, M., Patel, A.K., Shah, A.V., Raval, J., Rajpara, N., Joshi, M. & Joshi, C.G. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. *Sci of The Total Envi* **746**, 141326, doi:10.1016/j.scitotenv.2020.141326 (2020).

[22] Ahmadi, T.K.F.H. Ecology of some aquatic fungi recovered from water and submerged mud in different aquatic habitats within Sulaimani Province-Kurdistan region of Iraq. *The Egyp J of Exper Bio (Botany)* **7**, 103, (2015).

[23] Prasad, K.N., Yang, B., Yang, S., Chen, Y., Zhao, M., Ashraf, M. & Jiang, Y. Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (Litchi sinensis Sonn.) seeds. *Food Chem* **116**, 1–7, doi:10.1016/j.foodchem.2009.01.079 (2009).

[24] Coker, W.C. Letters from the Collection of Dr. Charles Wilkins Short. *J of the Elisha Mitchell Sci Society* **57**, 98–168 (1941).

[25] Rattan, S.S., Muhsin, T.M. & Ismail, A.L.S. Aquatic fungi of Iraq: species of Dictyuchus and Calyptralegnia. *Sydowia* **31**, 112–121 (1978).

[26] Watanabe, T. Pictorial Atlas of Soil and Feed Fungi, Morphology of Cultured Fungi and Key to Species. CRC Press (2002).

[27] Yeoh, W.K., Ali, A. & Forney, C.F. Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. *Postharvest Bio and Tech* **89**, 56–58, doi:10.1016/j.postharvbio.2013.11.006 (2014).

[28] Beckmann, A., Meyer, R., Lewandowski, J., Markewitz, A. & Gummert, J. German heart surgery report 2020: the annual updated registry of the German society for thoracic and cardiovascular surgery. *The Thoracic and Cardiovascular Surgeon* **69**, 294–307, doi:10.1055/s-0041-1730374 (2021).

[29] Shin, S., Park, S., Park, M., Jeong, E., Na, K., Youn, H.J. & Hyun, J. Cellulose nanofibers for the enhancement of printability of low viscosity gelatin derivatives. *BioRes* **12**, 2941–2954 (2017).

[30] Hashemi, S.M., Rostami, R., Hashemi, M.K. & Damalas, C.A. Pesticide use and risk perceptions among farmers in southwest Iran. Human and Ecological Risk Assessment: *An Inte J* **18**, 456–470, doi:10.1080/10807039.2012.652472 (2012).

[31] Sukal, T.M., Ellis, M.D. & Dewald, J.P. Shoulder abduction-induced reductions in reaching work area following hemiparetic stroke: neuroscientific implications. *Expe Brain Res* **183**, 215–223 (2007).

[32] Pitt, J.I. & Hocking, A.D. Primary keys and miscellaneous fungi. *In Fungi and Food Spoilage*, 53–143 (2009).

[33] Fitri, A., Maulud, K.N.A., Pratiwi, D., Phelia, A., Rossi, F. & Zuhairi, N.Z. Trend of water quality status in Kelantan River downstream, Peninsular Malaysia. *J Rekayasa Sipil* **16**, 178–184, doi:10.25077/jrs.16.3.178-184.2020 (2020).

[34] Ren, W., Qiao, Z., Wang, H., Zhu, L. & Zhang, L. Flavonoids: promising anticancer agents. Medicinal Research Reviews **23**, 519–534, doi:10.1002/med.10033 (2003).

[35] Salihu, S.O. & Bakar, N.K.A. Modified APHA closed-tube reflux colorimetric method for TOC determination in water and wastewater. *Envi Monitoring and Assessment* **190**, 1–12 (2018).

[36] Wang, L., Ling, Y., Jiang, H., Qiu, Y., Qiu, J., Chen, H., Yang, R. & Zhou, D. AphA is required for biofilm formation, motility, and virulence in pandemic Vibrio parahaemolyticus. *Inte J of Food Micro* **160**, 245–251, doi:10.1016/j.ijfoodmicro.2012.11.004 (2013).

[37] Nolasco-Pérez, T.D.J., Cervantes-Candelas, L.A., Buendía-González, F.O., Aguilar-Castro, J., Fernandez-Rivera, O., Salazar-Castañón, V.H. & Legorreta-Herrera, M. Immunomodulatory effects of testosterone and letrozole during Plasmodium berghei ANKA infection. *Frontiers in Cellular and Infection Micro* **13**, 1146356 (2023).

[38] Wood, S.E. & Willoughby, L.G. Ecological observations on the fungal colonization of fish by Saprolegniaceae in Windemere. *J of Applied Eco* (pp. 737–749), doi:10.2307/2403930 (1986).

[39] Willoughby, L.G. & Pickering, A.D. Viable Saprolegniaceae spores on the epidermis of the salmonid fish Salmo trutta and Salvelinus alpinus. Transactions of the British Mycological Society **68**, 91–95, doi:10.1016/S0007-1536(77)80157-5 (1977).

[40] Almeida, J., Johnson, R.M., Corliss, H.L., Molnar, B.E. & Azrael, D. Emotional distress among LGBT youth: The influence of perceived discrimination based on sexual orientation. *J of Youth and Adolescence* **38**, 1001–1014 (2009).

[41] Chłodnicki, M. & Ciałowicz, K. Tell el-Farkha (Ghazala): preliminary report, 2006 (2008).

[42] Misra, J.C. & Chakravarty, S. Flow in arteries in the presence of stenosis. *J of Biomechanics* **19**, 907–918, doi:10.1016/0021-9290(86)90186-7 (1986).

[43] Nabi, M.N., Akhter, M.S. & Shahadat, M.M.Z. Improvement of engine emissions with conventional diesel fuel and diesel–biodiesel blends. *Bioresource Tech* **97**, 372–378, doi:10.1016/j.biortech.2005.03.013 (2006).

[44] Toma, J.J., Shekha, Y.A. & Al-Barzinji, Y.O.M. An Ecological Assessment for Water Quality of Some Water Bodies in Koysinjaq-Erbil, Iraq. *Al-Nahrain J of Sci* **21**, 119–129 (2018).

[45] Toma, J.J. Limnological study in Dokan Lake, Kurdistan Region of Iraq. *J of Envi Studies* **6**, 1–12 (2011).

[46] Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L. & Sullivan, C.A. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol Reviews* **81**, 163–182, doi:10.1017/S1464793105006950 (2006).

[47] Jana, A., Kumari, N., Nandi, P., Naik, S., Chatterjee, A., Jaisawal, G.K., Hayasaki, K. & Ricci, C. Broad-band X-ray observations of the 2018 outburst of the changing-look active galactic nucleus NGC 1566. Monthly Notices of the Royal Astronomical Society **507**, 687–703, doi:10.1093/mnras/stab2155 (2021).

[48] Scharsack, J.P. & Franke, F. Temperature effects on teleost immunity in the light of climate change. *J of Fish Bio* **101**, 780–796, doi:10.1111/jfb.15163 (2022).

[49] Obubu, J.P., Mengistou, S., Fetahi, T., Alamirew, T., Odong, R. & Ekwacu, S. Recent climate change in the Lake Kyoga basin, Uganda: An analysis using short-term and long-term data with standardized precipitation and anomaly indexes. *Climate* **9**, 179, doi:10.3390/cli9120179 (2021).

[50] Ongley, E.D., Xiaolan, Z. & Tao, Y. Current status of agricultural and rural non-point source pollution assessment in China. *Envi Pollution* **158**, 1159–1168, doi:10.1016/j.envpol.2009.10.047 (2010).

[51] Weng, L., Zhang, H., Chen, H., Song, Z., Hsieh, C. J., Daniel, L., Boning, D. & Dhillon, I. Towards fast computation of certified robustness for ReLU networks. *In Inte Conference on Machine Learning* 5276–5285, PMLR (2018).

[52] Sapkale, P. H., Singh, R. K. & Desai, A. S. Optimal water temperature and pH for development of eggs and growth of spawn of common carp (Cyprinus carpio). *J Appl Anim Res* **39**, 339–345, doi:10.1080/09712119.2011.620269 (2011).

[53] Flajšhans, M. & Hulata, G. Common carp-Cyprinus carpio. Genetic impact of aquaculture activities on native populations. In Corosetti, D., Garcia-Vasquez, E. & Veerspoor, E. (eds.) Sixth Framework plan of the EC, final *sci* report, 32–39 (2007).

[54] Pauly, D. & Froese, R. Comments on FAO's State of Fisheries and Aquaculture, or 'SOFIA'. Mar Policy **36**, 746–752, doi:10.1016/j.marpol.2011.10.021 (2010).

[55] Ali, H., Khan, E. & Sajad, M. A. Phytoremediation of heavy metals—concepts and applications. *Chemosphere* 91, 869–881, doi:10.1016/j.chemosphere.2013.01.075 (2013).

[56] Bonansea, S., Ernst, O. R. & Mazzilli, S. R. Baseline for Brassica carinata components of nitrogen-use efficiency in southern South America. *Agronomy* **13**, 412, doi:10.3390/agronomy13020412 (2023).

[57] Robert, M. F., Morin, S., Beaulieu, N., Gauthier, F., Chute, I. C., Barsalou, A. & MacLeod, A. R. DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. *Nat Genet* **33**, 61–65 (2003).

[58] Bauer, R. T. Shrimps: Their diversity, intriguing adaptations and varied lifestyles (Vol. 42). Springer Nature (2023).

[59] Heydarnejad, M. S. Survival and growth of common carp (Cyprinus carpio L.) exposed to different water pH levels. *Turk J Vet Anim Sci* **36**, 245–249, doi:10.3906/vet-1008-430 (2012).

[60] Mukhtar, S., Rana, W. & Mukhtar, S. The development and psychometric properties of the Impact of Covid-19 Scale (IC19-S): A factor analysis approach. Psychiatr Danub **34**, 226–232 (2022).

[61] Raes, D., Steduto, P., Hsiao, T. C. & Fereres, E. AquaCrop—the FAO crop model to simulate yield response to water: II. Main algorithms and software description. Agron J **101**, 438–447, doi:10.2134/agronj2008.0140s (2009).

[62] Fahad, K. K. & Shuhaib, Z. O. Cultivation of different densities of common carp (Cyprinus carpio L. 1758) in floating cages in the Euphrates River in Nasiriyah city. *Univ Thi-Qar J Agric Res* **10**, 16–24, doi:10.54174/utjagr.v10i2.129 (2021).

[63] Bhatnagar, A. & Singh, G. Culture fisheries in village ponds: a multi-location study in Haryana, India. *Agric Biol J North Am* **1**, 961–968 (2010).

[64] Amankwaah, D., Cobbina, S. J., Tiwaa, Y. A., Bakobie, N. & Millicent, E. A. B. Assessment of pond effluent effect on water quality of Asuofia Stream, Ghana. *Afr J Environ Sci Technol* **8**, 306–311 (2014).

[65] Al-Salman, M. Islamic regional economic integration: Challenges and opportunities. In Preliminary Proceeding of the International Seminar on Ways and Means to Establish Islamic Common Market, 180–197 (2000).

[66] Mangat, H. K. & Hundal, S. S. Salinity tolerance of laboratory reared fingerlings of common carp, Cyprinus carpio (Linn.) during different seasons. *Int J Adv Res* **2**, 491–496 (2014).

[67] Alikunhi, K. H. Fish culture in India. Farm Bull **20**, 1–144 (1957).

[68] Parida, M. M., Santhosh, S. R., Dash, P. K., Tripathi, N. K., Lakshmi, V., Mamidi, N., Shrivastva, A., Gupta, N., Saxena, P., Babu, J. P. & Rao, P. L. Rapid and real-time detection of Chikungunya virus by reverse transcription loop-mediated isothermal amplification assay. *J Clin Microbi* **45**, 351–357, doi:10.1128/jcm.01734-06 (2007).

[69] Rao, R. V., Savsani, V. J. & Vakharia, D. P. Teaching–learning-based optimization: a novel method for constrained mechanical design optimization problems. *Comput Aided Des* **43**, 303–315, doi:10.1016/j.cad.2010.12.015 (2011).

[70] Furnas, M. J. & Mitchell, A. W. Photosynthetic characteristics of Coral Sea picoplankton (< 2 μm size fraction). *Biol Oceanogr* **5**, 163–182 (1988).

[71] Kappelman, L., McLean, E., Johnson, V. & Torres, R. The 2015 SIM IT Issues and Trends Study. *MIS Q Exec* **15**, 1 (2016).

[72] Davidson, N. C. How much wetland has the world lost? Long-term and recent trends in global wetland area. *Mar Freshw Res* **65**, 934–941, doi:10.1071/MF1417 (2014).

[73] Muammar, O. M. Future of gifted education: A talent development platform. Kurdish Stud **12**, 564–572, doi:10.53555/ks.v12i4.3006 (2024).

[74] Glewa, S. M. & Al-Alwani, M. Evaluation of the effect of solid waste leachate on soil at Hilla city. *J Babilon Univ* **3**, 894–906 (2013).

[75] Al Hadithi, T. S., Shabila, N. P., Al Tawil, N. G. & Othman, S. M. Demographic transition and potential for development: the case of Iraqi Kurdistan. *EMHJ-Eastern Mediterr Health J* **16**, 1098–1102 (2010).

[76] Zhang, W. R., Wang, K., Yin, L., Zhao, W. F., Xue, Q., Peng, M., Min, B. Q., Tian, Q., Leng, H. X., Du, J. L. & Chang, H. Mental health and psychosocial problems of medical health workers during the COVID-19 epidemic in China. Psychother Psychosom **89**, 242–250 (2020).

[77] Haque, M. S., Uddin, S., Sayem, S. M. & Mohib, K. M. Coronavirus disease 2019 (COVID-19) induced waste scenario: A short overview. *J Environ Chem Eng* **9**, 104660 (2021).

[78] Ngatia, L., Grace III, J. M., Moriasi, D. & Taylor, R. Nitrogen and phosphorus eutrophication in marine ecosystems. *Monit Mar Pollut* **1**, 1–17 (2019).

[79] Coyle, D., Nidaw, E., Getachew, H., Payne, S. R. & Subramaniam, R. Paediatric urology in Sub-Saharan Africa: challenges and opportunities. *BJU Int* **130**, 277–284, doi:10.1111/bju.15852 (2022).

[80] Guo, Z. Y. & Li, Z. X. Size effect on single-phase channel flow and heat transfer at microscale. Int J Heat Fluid Flow **24**, 284–298, doi:10.1016/S0142-727X(03)00019-5 (2003).

[81] Hassan, F. M. & Shaawiat, A. O. Application of diatomic indices in lotic ecosystem, Iraq. *Glob J Appl Phycol* **4**, 381–388 (2015).

[82] Lynn, R. & Mikk, J. National IQs predict educational attainment in math, reading, and science across 56 nations. Intelligence **37**, 305–310, doi:10.1016/j.intell.2009.01.002 (2009).

[83] Hejazy, M., Norouzi, R., Abdi, F. & Javid, F. The impact of aquaculture activities on nitrogenous and phosphorous pollution of water resources in northern Iran. *Arabian J Geosci* **16**, 255 (2023).