

Studies on Biochemical and Enzymatic Changes in Freshwater Fish *Xenentodon Cancila* Infected With Internal Parasite *Philometra Pellucida* and Analysis of Heavy Metals in the Water

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Author's Contributions

All of the authors worked together to complete this project. The study was developed by the first author, who also produced the protocol and the original draught of the publication. The study's analyses were overseen by the second and third authors. The literature searches were handled by the second author. The final manuscript was read and approved by all writers.

Abstract

Parasites present in fishes have been reported to severely change the status of biochemical and antioxidants in them. Among nematode parasite, *Philometra pellucida* is commonly found in freshwater fish, *Xenentodon cancila* is causing a considerable damage to fish health. The aim of this study was to investigate the biochemical and enzymatic changes in muscle, gut, liver and gill tissues of *Xenentodon cancila* parasitized by *Philometra pellucida*. The present result showed that the biochemical parameters including carbohydrate, protein and lipid are decreased in infected fish. The enzymatic manifestation of the infected fishes showed marked increase in the content of protease, Superoxide Dismutase, glutathione, glutathione peroxidase and lipid peroxidation concentration in infected fish. The heavy metals like Barium, Boron, Copper, Manganese, Selenium, Silver, Zinc, Cadmium, Lead, Chromium, Molybdenum, Nickel and Arsenic were analysed and aluminium is identified in a trace amount.

Keywords: Biochemical assays, antioxidant enzymes, nematode parasite, Superoxide dismutase, heavy metals

1. Introduction

Parasites are a significant concern to freshwater and marine fishes all-around the world, and are of specific importance in the tropics [1,2]. The parasites affect the fish biology, behaviour, nutrient devaluation, immune capability, blindness, morbidity, mortality, growth and fecundity reduction [3] and mechanical injuries depending on the parasite species and load [4].

Commercially important species of fishes are infected by parasites, which may have considerable impact on physiological and biochemical processes in the host organisms and harmful for their health. Parasites are widely distributed in water bodies, which are the main recipients of wastewaters, containing chemicals and pathogens, and the deleterious effects of them are well known [5]. The interactions between parasites, pathogens and chemicals possess a high potential for accumulation or synergetic effects. The roles, functions and life-styles of parasites help to characterize an ecosystem [6,7].

Hemato-biochemical indices have been employed in effectively monitoring the responses of organisms to stressors and thus its health status under such adverse conditions. In general, haematological tests are helps to establish normal health status and to diagnose diseases caused by various factors including parasitic infections, nutrition and pollution, genotoxic effect of pollutants, environmental stress and heavy metals in human and veterinary science [8]. Therefore, the changes associated with biochemical parameters due to various parasites found a database, which could be helped in diagnosis of diseases and in leading the application of the treatment or preventive measures [9].

Xenentodon cancila (freshwater garfish) is a needlefish found in freshwater and brackish habitats in South and Southeast Asia. It is rich with vitamin-A which plays an important role in preventing child blindness. The fish distributed in major area of South Asia and thus give a global consequence with its taste and nutrition [10,11].

Philometrid nematodes are commonly present in freshwater, brackish-water and marine fishes, occurring frequently in commercially important wild or cultured fish hosts. Most of the females are large- sized due to some morphological and biological peculiarities [12]. Most nematode species infect the intestinal tract [13]. The infected fish lost their intestinal contents and nutrients because the parasite damage the tissues. Large numbers of parasites lead to complete destruction of the intestinal mucosa and death of the fish. Fluid filled spaces were distributed on the inner surface of the body. Thus the parasitic infection causes biochemical and enzymatic changes in the host fish.

This work therefore, aimed at investigating the impact of parasites on the biochemical and enzymatic characteristics of freshwater fish *Xenentodon cancila*.

2. Materials and Methods

2.1. Collection of fish samples

The freshwater fish *Xenentodon cancila* were obtained from four dams Pechiparai, Perunchani, Chittar-1 and Chittar-2 of Kanyakumari District, Tamil Nadu, India. The sampling was carried out during August-September 2020. Ten fish from each dam were examined and assessed biochemical and enzymatic changes. Some fishes were infected with internal nematode parasite *Philometra pellucida*. Gill, liver, gut and muscle of each fish were dissected out and weighed.

2.2. Parasitological examination

Parasitological examination was carried out for the detection and identification of the parasite *Philometra pellucida* in the body cavity and internal organs of the fish.

2.3. Biochemical analysis

Biochemical tests were performed for determination of the carbohydrate, total protein and lipid of different organs of the fish *Xenentodon cancila*. The estimation of carbohydrate content was carried out by Carrol et al. [14], total protein concentration was estimated by Lowry's method [15] and lipid content was measured by Frings et al. [16].

2.4. Enzymatic assays

Protease activity is detected by caseinase assay method [17], α -Amylase activity was determined by Bernfeld [18], superoxide dismutase activity was determined according to the method of Mc Cord and Fridovich [19], reduced glutathione in the tissue was determined according to the method of Moron et al. [20], glutathione peroxidase activity was determined according to the method of Hafemann et al. [21] and the level of lipid peroxidation was measured as malondialdehyde (MDA) according to the method of Ohkawa et al. [22].

2.5. Heavy metals detection

Four samples of water from the four dams namely, Pechiparai, Perunchani, Chittar-1 and Chittar-2, the sources of fish collection were taken in the four mentioned flask one-liter volume capacity after its rinsing several times with distilled water and sterilized in hot air

oven at 180 °C/hour. The collected water sample bottles were labelled with the locality, date, and time of collection. Chemical examinations of these samples were done to estimate some heavy metals including (aluminium, barium, copper, manganese, selenium, silver, zinc, cadmium, lead, chromium, molybdenum, nickel, arsenic and mercury) according to Chapman and Pratt [23].

3. Result and discussion

3.1. Biochemical in freshwater fish *Xenentodon cancila*

The evaluation of animal's health, especially wild fish populations has required the development of biological markers of their status in different levels of their biological organization. Sensitivity or resistance of the wild life to various stressors depends on many exogenous and endogenous factors and for the first time, on the status of defense mechanisms, which protect an organism against diseases and environmental pollution caused anthropogenic impact. Generally, various biochemical and enzymatic parameters are used for the evaluation of fish health. They often reflect physiological status of the animal and the influence of certain biotic (pathogens, parasites, disease state, etc) and abiotic factors (anthropogenic pollution, habitat degradation, etc) on it [24,25]. Among them biochemical characteristics are used widely as early warning indicators of changes in health status.

The quantitative values of biochemical estimation in freshwater fish, *Xenentodon cancila* is shown in the Table 1; Fig. 1. Carbohydrate and protein content was higher in uninfected fish than infected fish organs gut, liver, gill and muscle. Lipid content was higher in uninfected fish muscle and gut, but lower in uninfected liver and gill. Carbohydrate content was lower in infected muscle (33.5 ± 0.35), gut (34.9 ± 0.64), liver (28.4 ± 0.35) and gill (28.4 ± 0.37) as compared to uninfected fish organs (33.5 ± 0.35 , 34.9 ± 0.64 , 28.4 ± 0.35 and 28.4 ± 0.37) respectively. Likewise, the protein content was higher in uninfected muscle, gut, liver and gill (61.3 ± 1.13 , 41.2 ± 0.74 , 29.5 ± 0.51 and 30.8 ± 1.27) than infected fish organs (22.1 ± 0.88 , 24.8 ± 1.20 , 18.4 ± 0.48 and 19.2 ± 0.48) respectively. Unfortunately, the lipid content of the infected fish liver (4.8 ± 0.33) and gill (7.7 ± 0.48) was increased than uninfected fish liver (2.0 ± 0.14) and gill (3.0 ± 0.21). This indicates the significant difference between infected and uninfected fishes.

Table 1. Biochemical parameters of freshwater fish, *Xenentodon cancila*

Organs		Biochemical Tests		
		Carbohydrate (mg/g)	Protein (mg/g)	Lipid (mg/g)
Muscle	Uninfected fish	36.1±0.88	61.3±1.13	5.5±0.36
	Infected fish	33.5±0.35	22.1 ± 0.88	3.0±0.14
Gut	Uninfected fish	40±0.14	41.2±0.74	6.0±0.28
	Infected fish	34.9±0.64	24.8±1.20	4.0±0.14
Liver	Uninfected fish	35.1±0.73	29.5±0.51	2.0±0.14
	Infected fish	28.4±0.35	18.4±0.48	4.8±0.33*
Gill	Uninfected fish	31.4±0.37	30.8±1.27	3.0±0.21
	Infected fish	28.4±0.37	19.2±0.48	7.7±0.48*

Each value is mean ± SD of observations, * indicates significant

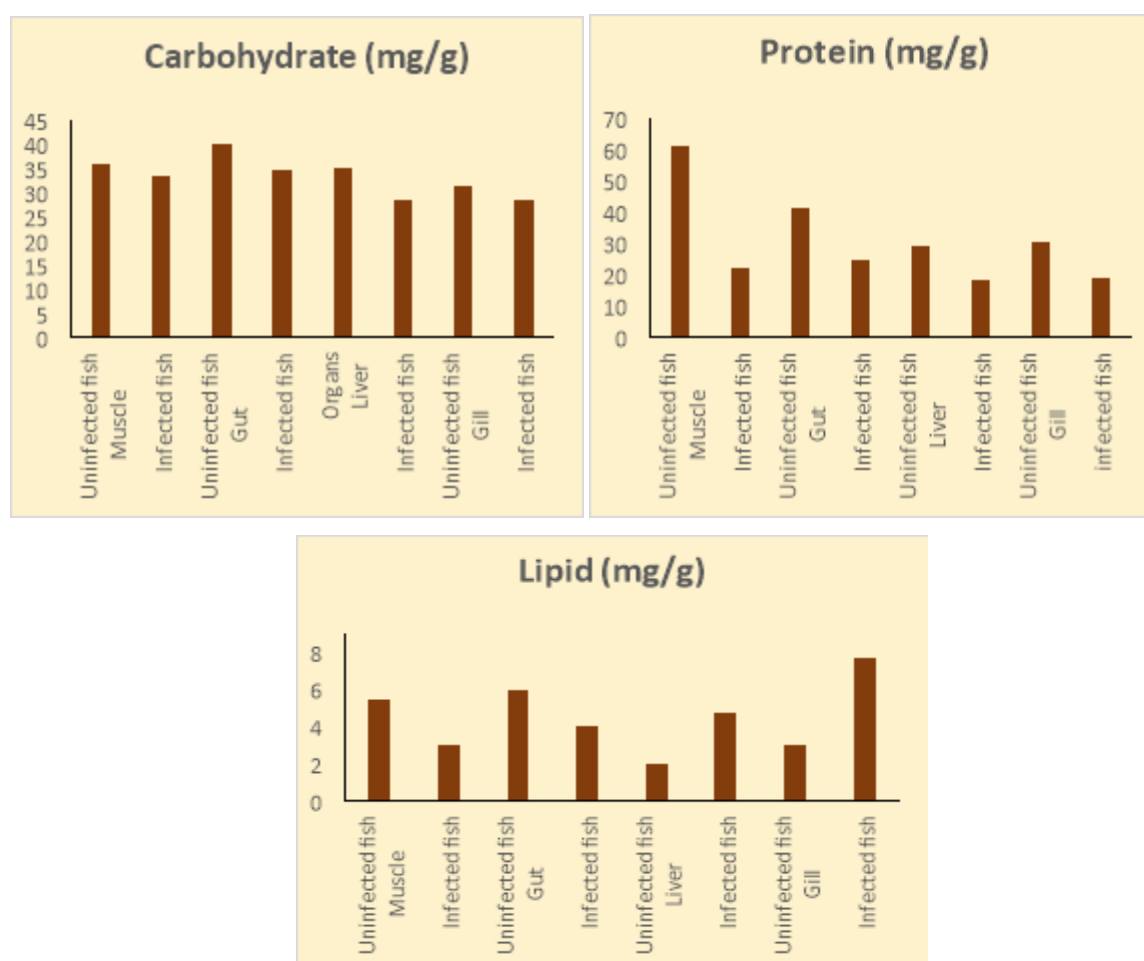


Figure 1. Carbohydrate, Protein and Lipid content of freshwater fish, *Xenentodon cancila*

During the present study, freshwater fish *X. cancila* infected with internal nematode parasite *P. pellucida* showed the decline in biochemical parameters that is carbohydrate, protein and lipid content of the infected fish compared to uninfected fish. These results are in accordance with that mentioned by Hassan et al. [26] where they recorded a reduction in total protein and carbohydrate in liver and muscle in the case of helminthes either infecting fish *Epinephelus summana*. Our results also agree with the data of Rajaram et al. [27] reported the carbohydrate and lipid content was lower in infected fish with crustacean parasite Cymothoid isopods. But significant increase of lipid level was observed in liver and gill it is also supported by a study that hydroxyl lipid levels in liver were significantly higher in diseased fish than in normal fish [28].

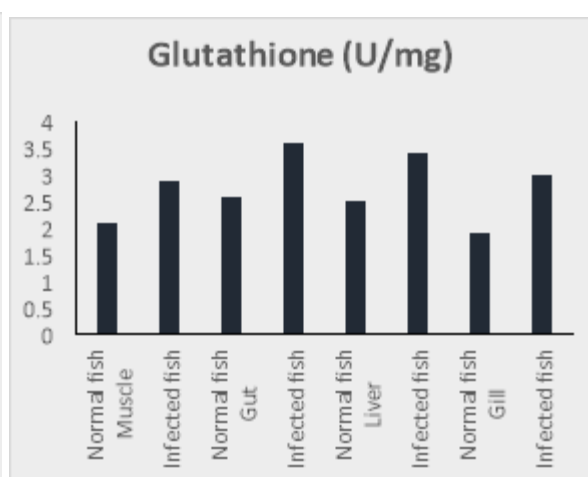
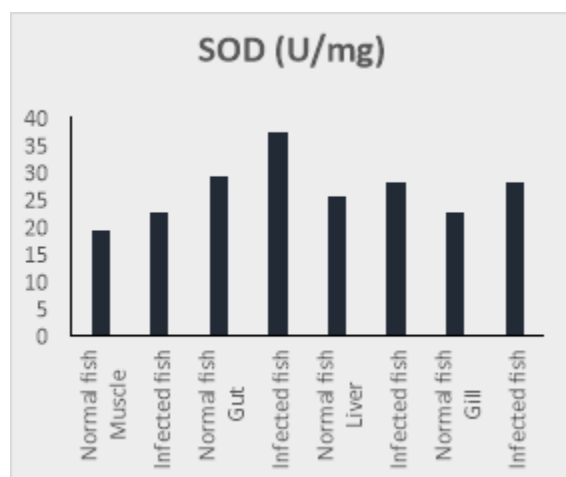
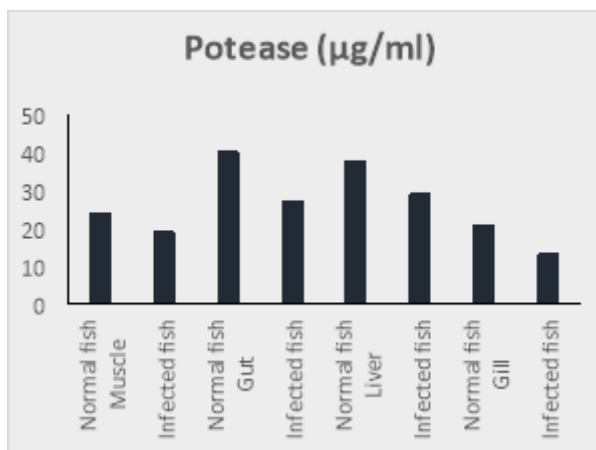
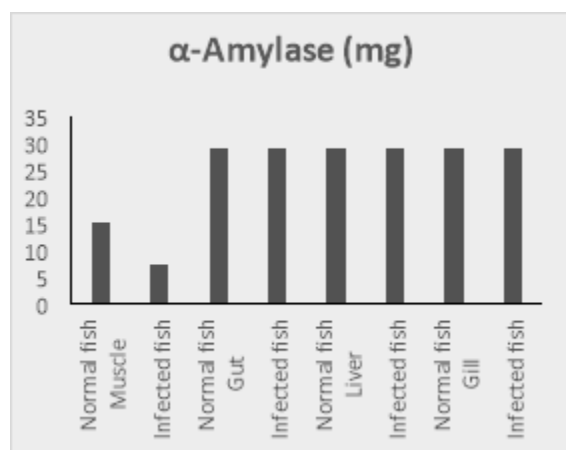
3.2. Enzymatic analysis of freshwater fish, *Xenentodon cancila*

The effect of nematode parasite *P. pellucida* on the level of α -amylase, protease, Superoxide Dismutase, glutathione, glutathione peroxidase and lipid peroxidase content in muscle, gut, liver and gill in comparison with infected and uninfected fish *X. cancila* is given below.

The level of α -amylase higher in uninfected fish muscle (15.1 ± 0.6) and gut (29.1 ± 0.54) than infected fish muscle and gut (7.5 ± 0.35 , 19.0 ± 0.74) respectively and lower in uninfected fish liver (18.2 ± 0.96) and gill (24.2 ± 1.00) than uninfected fish liver and gill (22.2 ± 0.92 , 28.3 ± 0.99) respectively. Presence of infection in the fish due to nematode caused decreased in protease content in muscle (19.1 ± 0.54), gut (27.2 ± 0.76), liver (29.1 ± 0.54), gill (13.2 ± 0.45) of infected fish as compared to uninfected fish organs (24.2 ± 0.48 , 40.2 ± 0.45 , 37.9 ± 1.30 and 20.9 ± 0.60) respectively. The SOD activities observed in the muscle (19.2 ± 0.48), gut (29.2 ± 0.76), liver (25.4 ± 0.35) and gill (22.4 ± 0.36) were lower in uninfected fish as compared to respective organs (22.5 ± 0.88 , 37.1 ± 0.73 , 27.9 ± 0.67 and 28.1 ± 0.88) of the infected fish. Notably, Glutathione levels increased in muscle (2.9 ± 0.25), gut (3.6 ± 0.37), liver (3.4 ± 0.43) and gill (3.0 ± 0.44) of infected fish as compared to normal fish organs (2.1 ± 0.21 , 2.6 ± 0.43 , 2.5 ± 0.38 and 1.9 ± 0.10) respectively. Lipid peroxidation levels in muscle, gut, liver and gill of both infected and uninfected fish are given in the Table 2 and Fig. 2. Increased level of lipid peroxidation was observed in muscle (34.8 ± 1.24), gut (55.6 ± 1.08), liver (53.6 ± 0.83) and gill (45.1 ± 0.73) of infected fish as compared to normal fish organs (25.0 ± 0.70 , 25.0 ± 0.71 , 28.1 ± 0.64 and 20.9 ± 0.81) respectively.

Table 2. Enzymatic analysis of freshwater fish, *Xenentodon cancila*

Organs		Enzymatic assays					
		α -Amylase (mg)	Protease (μ g/ml)	SOD (U/mg)	Glutathione (U/mg)	Glutathione peroxidase (nmol/mg)	Lipid peroxidation (nmol/mg)
Muscle	Normal fish	15.1 $\pm$ 0.6	24.2 $\pm$ 0.48	19.2 $\pm$ 0.48	2.1 $\pm$ 0.21	26.1 $\pm$ 0.54	25.0 $\pm$ 0.70
	Infected fish	7.5 $\pm$ 0.35	19.1 $\pm$ 0.54	22.5 $\pm$ 0.88	2.9 $\pm$ 0.25	32.0 $\pm$ 0.70	34.8 $\pm$ 1.24
Gut	Normal fish	29.1 $\pm$ 0.54	40.2 $\pm$ 0.45	29.2 $\pm$ 0.76	2.6 $\pm$ 0.43	20.1 $\pm$ 0.81	25.0 $\pm$ 0.71
	Infected fish	19.0 $\pm$ 0.74	27.2 $\pm$ 0.76	37.1 $\pm$ 0.73	3.6 $\pm$ 0.37	24.2 $\pm$ 0.96	55.6 $\pm$ 1.08
Liver	Normal fish	18.2 $\pm$ 0.96	37.9 $\pm$ 1.30	25.4 $\pm$ 0.35	2.5 $\pm$ 0.38	20.2 $\pm$ 0.48	28.1 $\pm$ 0.64
	Infected fish	22.2 $\pm$ 0.92	29.1 $\pm$ 0.54	27.9 $\pm$ 0.67	3.4 $\pm$ 0.43	25.1 $\pm$ 0.88	53.6 $\pm$ 0.83
Gill	Normal fish	24.2 $\pm$ 1.00	20.9 $\pm$ 0.60	22.4 $\pm$ 0.36	1.9 $\pm$ 0.10	22.0 $\pm$ 0.70	20.9 $\pm$ 0.81
	Infected fish	28.3 $\pm$ 0.99	13.2 $\pm$ 0.45	28.1 $\pm$ 0.88	3.0 $\pm$ 0.44	27.0 $\pm$ 0.70	45.1 $\pm$ 0.73



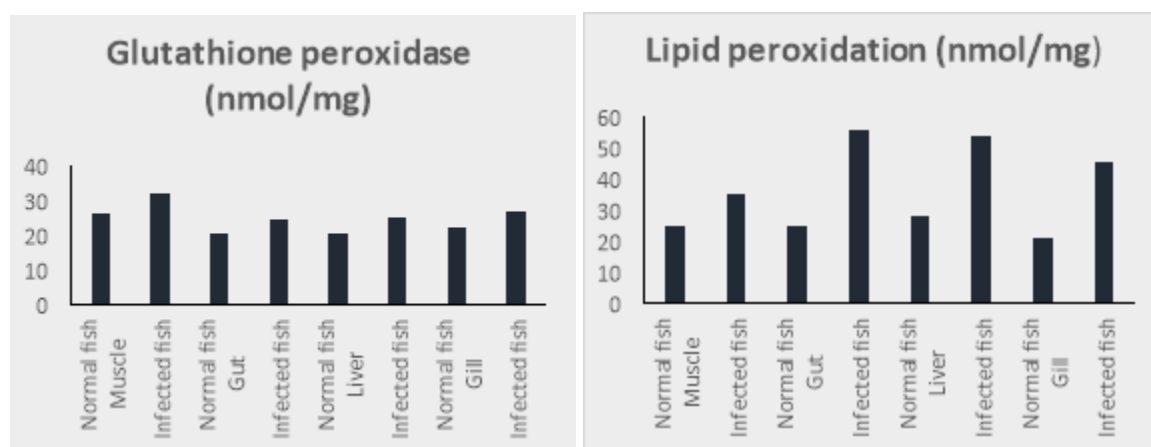


Figure 2. Enzymatic analysis of freshwater fish, *Xenentodon cancila*

In living organisms, oxidative stress is caused by a variety of endogenous and external sources. Organisms are adapted various mechanisms that protect against oxidative stress caused by a variety of biotic and abiotic agents, including parasite infection, including the induction of antioxidant enzymes SOD, catalase CAT, peroxidase PER, and glutathione related enzymes (GR, glutathione peroxidase (GP), and GST) [29]. Infections with parasites have been shown to alter the biotransformation system [30], immunological response [31], and antioxidant activities [30,32]. Antioxidant and detoxifying enzymes have long been utilised as indicators of oxidative stress in fish and invertebrates subjected to xenobiotics in a laboratory setting or in a polluted environment [33,34]. Invasion of a stressed organism can cause the generation of reactive oxygen species (ROS).

The results of the analysis demonstrated that parasite invasion has a considerable impact on the antioxidant state of whiting tissues. Our findings corroborate those of several other researchers who have found that parasite invasion alters antioxidant enzymatic activity in fish. However, the antioxidant system's reaction in parasitized fish varies depending on parasite species, examined tissues, and enzyme specificity. Furthermore, pollution may influence the antioxidant response of parasitized fish [35].

The increased activity of SOD, glutathione, glutathione peroxidase, and lipid peroxidation in the infected host's muscle, gut, liver, and gills in the current study indicates enhanced oxidative stress caused by parasite infection. Antioxidant activities have been documented to be altered by a variety of conditions [36], including parasite infections that disrupt the host's metabolic pathways [37]. As a result, the rise or decrease in several antioxidant enzymes in infected *X. cancila* muscle, gut, liver, and gill during the current investigation could also be connected to the parasite-induced metabolic activity modulation.

The infected fish's oxidative stress is modulated differently in different organs due to changed metabolic pathways. Thus according Tabatabaie and Floyd [38], negative feedback from an overabundance of substrate can reduce enzyme activity, or it could be damaged by oxidative modification. Reduced activity of -amylase in muscle and gut, as well as protease in muscle, gut, liver, and gill of infected fish, is perhaps one of the explanations for this.

The presence of *Clinostomum detrunctum* in the organs of freshwater fish caused oxidative stress and increased membrane damage in the skeletal muscle of infected fish [39], which is consistent with our findings in muscle, gut, liver, and gill. The regulation of oxidative stress in *X. cancila* caused by parasitic infection with *P. pellucida* is a sign of the infected host's oxidative defence against the invading nematode parasites. It is hypothesised that parasitic infection causes an increase in antioxidant enzyme activity in the hosts, which would be a form of parasitic approach for reducing the host's oxidative response, and hence aids in the parasite's long-term survival inside the host [40,41].

3.3. Mineral content in different water samples

Heavy metal exposure affects fish with parasite diseases, according to a review of the literature. The presence of heavy metals in water samples from three reservoirs necessitated a trial. Heavy metals such as barium, boron, copper, manganese, selenium, silver, zinc, cadmium, lead, chromium, molybdenum, nickel, and arsenic were not found in any of the reservoirs' water samples. However, aluminium was identified in all of the reservoirs, with chittar-2 having the highest concentration at 0.042 mg/l (Table 3).

Table 3: Mineral content in different water samples

Minerals	Water Samples			
	Pechiparai	Perunchani	Chittar-1	Chittar-2
Aluminum (Al)	0.006mg/l	0.006 mg/l	0.028 mg/l	0.042 mg/l
Barium (Ba)	-	-	-	-
Boron (Br)	-	-	-	-
Copper (Cr)	-	-	-	-
Manganese (Mn)	-	-	-	-
Selenium (Se)	-	-	-	-
Silver (Ag)	-	-	-	-

Zinc (Zn)	-	-	-	-
Cadmium (Cd)	-	-	-	-
Lead (Pb)	-	-	-	-
Chromium (Cr)	-	-	-	-
Molybdenum (Mo)	-	-	-	-
Nickel (Ni)	-	-	-	-
Arsenic (As)	-	-	-	-
Mercury (Hg)	-	-	-	-

To link the presence of heavy metals to parasite diseases, researchers discovered that even very small amounts of heavy metals are hazardous to fish in water. Heavy metals damage fish, and these fish are eaten by a huge portion of the population. As a result, human health is at jeopardy, and water toxicity must be closely monitored [42].

4.Conclusion

As might be evident from the present study that the pechiparai, perunchani, chittat 1 and 2 possesses better water quality status under the study period since all the Bio-chemical analyses indicate the values within the maximum permissible limits for drinking water as recommended by WHO. Also the study indicates that infection of the nematode parasite can change the biochemical and antioxidant enzymes composition of fish species. The heavy metals due to the pollutants are also have influence on fish infection.

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