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Pathological effect of water polluted with heavy metals and fish parasites on *Carangoides bajad* (Forsskål, 1775) from the Red Sea coast of Rabigh region, Saudi Arabia

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Pollution of the ecosystem is a source of concern and wide attention globally. In this study, samples of water was collected and 50 fish samples of Carangoides bajad to assess the rate of water pollution of the coastal area of Rabigh on the coast of the Red Sea and that is due to its closeness to industrial activities. Concentrations of some heavy metals were assessed and was estimated as follows Zn > Fe > Pb (81 > 15.1 > 12.6 µg/l, respectively) using atomic absorption spectrophotometer (Model AA-7000, Shimadzu, Japan). Using a light microscope, different types of endoparasites and ectoparasites were recognized in the chosen infected fish; in addition, a histological examination was conducted for some targeted organs (gills, intestines, liver). The results revealed that 40 fish were infected with subclass Copepoda Hatschekia (intensity 2); digenean parasites Bucephalus margaritae (intensity 4.36), Plagioporus ira (intensity 3.09), and Tergestia bengalensis (intensity 2.33); and Anisakis physeteris (intensity 12.57). The results revealed many histopathological changes which demonstrate the presence of potential risks on consumers due to pollution of the aquatic ecosystems. Therefore, continuous monitoring of these pollutants is an essential matter to ensure the safety of aquatic organisms and humans that rely on these aquatic resources.

Keywords: water pollution; heavy metals; parasite; gills; liver; intestines.

1. INTRODUCTION

Environmental concerns raised by human activities that do not consider the nature of ecological systems are gaining considerable research attention globally. Although we rely on aquatic resources in several industries such as the manufacturing, food, and entertainment industries, these activities pollute the environment by dumping industrial waste, combustion products, and other substances with heavy metals that increase toxicity (Kleinertz and Palm, 2013).

Parasites are key elements in the aquatic ecosystem that aid basic ecological processes such as the composition of food webs, biodiversity and productivity of the ecosystem (Poulin, 1999; Marcogliese, 2004). Therefore, an ecosystem that has good performance and flexibility (Costanza and Mageau, 1999) is a system enriched with various types of parasites (Marcogliese, 2005; Hudson et al. 2006).

Parasites are important components of ecosystem and play a significant role in biodiversity. They provide valuable information about their hosts and the environment in which they exist. Studies on *Hatschekia* (Poche, 1902) in marine fish from various regions of the world have received considerable attention since the turn of the century. The family Hatschekiidae (Kabata, 1979) (Copepoda: Siphonostomatoida) is one of the major gill parasitic groups in marine fish. *Hatschekia* (Poche, 1902) is the largest genus of this family with 176 valid species recognized by the World Register of Marine Species (Walter and Boxshall, 2019). Although these copepods produce a small amount of eggs, they are capable of infecting several fish species and often accumulate on their host in large numbers (Jones, 1998). The parasites on the gills of fish can cause extensive gill damage and severe hemorrhaging with inflammation and exsanguination caused by their attachment and feeding habits (Lester and Hayward, 2006).

Digeneans with their heteroxenous life cycle have not only a definitive host but also primary (mollusk) and secondary (fish) hosts. *Plagioporus* is a genus of Trematoda class parasites and subclass Digenea. The species of this genus, such as *Plagioporus ira* (Yamaguti, 1940), occurs in both marine and freshwater teleosts around the world, and was first identified in *Choerodon azurio*. Parasites transmitted through the food chain alter their hosts to increase virulence, and thus, they enhance parasitic transmission. Increase in virulence enables the parasite to increase its reproductive output. This increases their prevalence and abundance rates in addition to their pathogenicity (Price, 1980).

The digenetic trematode, *Bucephalus margaritae,* was first described by Ozaki & Ishibashi, (1934); it was also previously identified in the intestine of *Carangoides bajad* (Bakhraibah,1999). Linnaeus, (1758) showed that *Bucephalus* larvae in *Perna perna* mussel on the coast of Santa Catarina caused severe effects on the culture and commercial production of mussels because of the elevated degrees of pathogenicity of the larvae (Da Silva et al. 2002). Bucephalosis destroys the reproductive tissues of the host and disables its gametogenesis leading to its castration (Coustau et al. 1990) and possibly, its death (Da Silva et al. 2002). As mentioned by Nahhas et al. (2006), the diagnostic character for *B. margaritae* is the presence of seven tentacles, each containing two projections, one big and basal and the other small and distal. *Bucephalus* can be found worldwide in tropical and subtropical waters such as in the Arabian Gulf off the coast of Kuwait, Red Sea, Arabian Sea, Philippines, Hawaii, India, and Japan (Nahhas et al. 2006; Amato, 1982; Chinchilla et al. 2006).

Tergestia bengalensis was first discovered by Gupta and Singh, (1985) and classified under the genus *Tergestia*. It has been previously reported in *C. bajad* species as well (Bakhraibah,1999). Although this genus is found frequently in the intestines of hosts and is usually present in large numbers, only a few were present in our host fish. Further, there is a lack of sufficient literature on the characteristics and effects of this parasite.

Anisakis physeteris (Baylis, 1923) is a species of the parasitic nematode of the subclass Digenea, of family Anisakidae, which is commonly found in the intestines and liver of its hosts. *Anisakis physeteris* is not host specific and can be transmitted through the ingestion of other infected crustaceans. Adult parasites usually appear in pinnipeds and cetaceans. This parasite is temperature dependent; under low environmental temperatures, there is a decrease in the number of larvae penetrating the fish. Larval stages in fish tend to be more prevalent in areas where various hosts are available in large numbers such as in inshore waters (Smith and Wootten, 2012).

In recent years, there has been an increased focus on identifying the correlation between parasitism and pollution, studying the role of parasites as biomarkers of pollutants and the health of the ecosystem, and the interaction between parasites and their hosts. Parasitism increases the ability of the host to intake toxic pollutants. An increase or decrease in pollutants can cause an increase or a decrease in the prevalence of the parasites, thus, making parasites effective biomarkers for pollution in an environment with heavy metals (Goater et al. 2013; Sures et al. 2017).

Studies have focused on the potential roles of parasites as biomarkers of the quality of water and their suitability as biomarkers of the quality of the environment (Vidal-Martínez et al. 2010); other studies have focused on the use of fish parasites as good organisms for the biological monitoring of heavy metal pollution in the aquatic environment (Sures et al. 2017). The aquatic environment can be studied either directly, through regular monitoring for quality markers of the water, or indirectly, by using biological factors such as fish parasites (Bayoumy et al. 2015).

The quality of water is considered an important factor that helps determine the prevalence of parasites (Authman et al. 2008); it can enhance the presence of the parasites in an increasing manner in aquatic animals and especially in fish (Oros and Hanzelová, 2009). External parasites are directly in contact with the environment which makes them a biomarker for pollutants that directly affect them, reducing their vitality and population rate (Pietrock et al. 2008; Khalil et al. 2014).

There are two routes by which fish can be exposed to heavy metals: one is the presence of chemicals in water, where the dissolved contaminants are transferred through biological membranes and ionic exchange in the gills. The second is exposure through the intestines caused by ingesting food and sediment particles (Burger et al. 2002). The liver and gill tissues are used for histological examinations as they exhibit high metabolic activity caused by a higher accumulation of heavy metals (Barson et al. 2014) A diverse group of living contaminants is inspected to assess their ability as biomarkers for different types of aquatic pollution. The relationship between pollution and parasites in living organisms, and their potential roles as biomarkers of the quality of water have received increasing research attention in the last couple of decades. In this study, we detected the concentration of heavy metals (Fe, Pb, and Zn) to assess the state of pollution in the coastal area of Rabigh on the Red Sea coast, to identify the relationship between the heavy metal concentration and parasites and to determine the potential risks for fish considered as biomarkers of the pollution level of the coastal water of Rabigh.

Food is a vital source for heavy metals accumulation in biological waters (Clearwater et al. 2000). Heavy metals are transported through the food chain, which affects the health of the consumers. An accumulation of heavy metals in humans can cause chronic and acute damages in the local communities, thereby reducing growth and reproductive ability (Schulz and Martins-Junior et al. 2001). This study aims to determine and investigate the concentration of heavy metals and its relationship with parasites in the waters in the Rabigh region. This region is located near petrochemical industries and effluent producing industries and is, thus, prone to heavy metal pollution (Nayebare et al. 2017).

2. MATERIALS AND METHODS

2.1. Study Area

The study area is located on the western Red Sea coast of the Rabigh region in the Kingdom of Saudi Arabia (Fig. 1). The coastal area of Rabigh, with its King Abdul Aziz Port is an important tourist attraction; it has petroleum refineries, a water and Electricity Company, cement factories, and consumption and food industries. Thus, it is important to study the environment of the coastal area of Rabigh. Further, coral reefs and other aquatic species in the Red Sea serve as main attractions for tourists.

Figure (1): A map demonstrating the Sea of Rabigh in which the samples were obtained from

2.2. Water Sampling

Water samples for the analysis were collected from the surface water of the study area (at a depth of 20 cm from the water surface to avoid floating substances), once a week for an entire month (in December, 2018). These water samples were collected in 1 L polyethylene bottles that were previously washed with acidic water (6N-HNO_3) and then with deionized water. The concentrations of Fe, Pb, and Zn in the sample water were analyzed by using an atomic absorption spectrophotometer (Model AA-7000, Shimadzu, Japan) while following the standard protocol (APHA, 2012).

2.3. Fish Samples

Samples of *Carangoides bajad* (Fig. 2) that belongs to family Carangidae were collected from the study area, once a week for a month. This species were selected owing to their economic importance and palatable taste. Two types of fishing nets—gills and trammel nets—were used for collecting the fish. These traps were fixed at multiple random points in the study area. Further, the total weight (g) and length (cm) of the fish were measured and recorded using a measuring tape and a weighing scale (Mojekwu and Anumudu, 2015).

Classification
Animalia **(Kingdom)**
Chordata **(Phylum)**
Vertebrata **(Subphylum)**
Gnathostomata **(Superclass)**
Pisces **(Superclass)**
Actinopterygii **(Class)**
Perciformes **(Order)**
Percoidei **(Suborder)**
Carangidae **(Family)**
Carangoides **(Genus)**
Carangoides bajad **(Species)**

Figure (2): A photograph of *Carangoides bagad* (Forsskål, 1775) fish sample captured from the study area

2.4. Parasitological Examination

A total of 50 fish of the *C. bajad* species were examined to study the endoparasites and ectoparasites that infect the fish. The gill cover was cut to extract the external parasites attached to it. The gills were then kept in a Petri dish with a 75% physiological solution and examined under a light microscope. If the parasites were attached to the gill filaments, the gill filaments and covers were separately placed in a physiological solution in the freezer for a couple of hours until the parasites fell to the bottom of the dish. These parasites were then pigmented by following the methods described by Carleton, (1957) and Hoffman, (1970), and they were later examined under a microscope. In addition, the internal organs of fish (stomach, abdominal cavity, intestines, testes, and ovaries) were dissected to extract the endoparasites. The parasites that were collected were then identified and classified based on the data provided by Yamaguti, 1953a; 1953a;1958; 1963; Yamaguchi, 1968; Ho et al. 1988; Kensley and Schotte, 1989.

Classification
Animalia **(Kingdom)**
Arthropoda **(Phylum)**
Crustacea **(Subphylum)**
Multicrustacea **(Superclass)**
Hexanauplia **(Class)**
Copepoda **(Subclass)**
Neocopepoda **(Infraclass)**
Podoplea **(Superorder)**
Siphonostomatoida **(Order)**
Hatschekiidae **(Family)**
Hatschekia **(Genus)**

Figure (3): A micrograph of *Hatschekia* Poche, 1902 found on the gills of *Carangoides bagad* (Forsskål, 1775)

Classification
Animalia **(Kingdom)**
Platyhelminthes **(Phylum)**
Rhabditophora **(Subphylum)**
Neodermata **(Superclass)**
Trematoda **(Class)**
Digenea **(Subclass)**
Plagiorchiida **(Order)**
Xiphidiata **(Suborder)**
Allocreadioidea **(Superfamily)**
Opecoelidae **(Family)**
Plagioporinae **(Subfamily)**
Plagioporus **(Genus)**
Plagioporus ira **(Species)**

Figure (4): A micrograph of *Plagioporus ira* (Yamaguti, 1940) in the intestines of *Carangoides bajad* (Forsskål, 1775)

Classification
Animalia **(Kingdom)**
Platyhelminthes **(Phylum)**
Rhabditophora **(Subphylum)**
Neodermata **(Superclass)**
Trematoda **(Class)**
Digenea **(Subclass)**
Plagiorchiida **(Order)**
Bucephalata **(Suborder)**
Bucephaloidea **(Superfamily)**
Bucephalidae **(Family)**
Bucephalinae **(Subfamily)**
Bucephalus **(Genus)**
Bucephalus margaritae **(Species)**

Figure (5): A micrograph of *Bucephalus margaritae* (Ozaki & Ishibashi, 1934) in the intestines of *Carangoides bajad* (Forsskål, 1775)

Classification
Animalia **(Kingdom)**
Platyhelminthes **(Phylum)**
Rhabditophora **(Subphylum)**
Neodermata **(Superclass)**
Trematoda **(Class)**
Digenea **(Subclass)**
Plagiorchiida **(Order)**
Bucephalata **(Suborder)**
Gymnophalloidea **(Superfamily)**
Fellodistomidae **(Family)**
Tergestiinae **(Subfamily)**
Tergestia **(Genus)**
Tergestia bengalensis **(Species)**

Figure (6): A micrograph of *Tergestia Bengalensis* (Gupta & Singh 1985) in intestines of *Carangoides bajad* (Forsskål, 1775)

Classification
Animalia **(Kingdom)**
Nematoda **(Phylum)**
Chromadorea **(Class)**
Chromadoria **(Subclass)**
Rhabditida **(Order)**
Spirurina **(Suborder)**
Ascaridomorpha **(Infraorder)**
Ascaridoidea **(Superfamily)**
Anisakidae **(Family)**
Anisakinae **(Subfamily)**
Anisakis **(Genus)**
Anisakis physeteris **(Species)**

Figure (7): A microscopic photograph of *Anisakis physeteris* (Baylis, 1923).

2.5. Histological Examination

Fish samples were dissected to extract the gills, intestines, and liver, and the histological changes of these organs were then assessed. Further, 3-mm-thick tissues from each organ were dipped in 10% Bouin's solution for 24 h. These tissues were then washed well with running water until the remaining formalin was removed. In the case of gills, around 0.5 cm of a gill arch was dipped in ethylene diamine tetra acetic acid (EDTA), a calcium extractant, for 2–3 days while renewing the solution each day. These gill arches were then transferred into a 70% alcohol solution.

Further, water was extracted from the tissue sections by passing it through a gradual series of alcohol concentrations, and then, the tissue was transferred into a xylene solution and dipped in paraffin wax. Later, thin transverse sections (T.S, 3 µm thick) of the intestines and liver tissues and longitudinal sections (L.S) of the gills were obtained using a rotary microtome; the sections were then pigmented with hematoxylin and eosin (HE).

3. RESULTS AND DISCUSSION

3.1. Estimation of heavy metal concentrations in water

In this study, the effect of industrial activities on heavy metal pollution such as Pb, Zn, and Fe were assessed from the surface seawater samples from the coastal area of Rabigh. Table (1) lists the concentrations of Pb, Zn, and Fe in the samples.

Our results revealed that the mean concentrations of heavy metals exceeded the global permissible limits for heavy metals in seawater, according to the Certified Reference Seawater Probe CASS-5 of the Institute for Environmental Chemistry, Canada. Many studies have focused on the coastal areas of the Red Sea that were severely stressed (e.g., ports) owing to the various activities in the coastal area (Dar et al. 2016; El-Taher et al. 2012). Heavy metals are dangerous pollutants owing to their toxic effect on plants, living organisms, and food. Many organizations have recommended the need to monitor the levels of trace elements in the aquatic environment (Younis et al. 2014; UNEP/FAO, 1996).

These metals are distinguished by their persistence, accumulation, and difficulty in metabolize in other intermediate compounds, as they do not degrade easily in the environment. Thus, they accumulate in the food chain and are a threat to humans as these metals are mutagens and highly cancerous (Raikwar et al. 2008).

Heavy metals enter the aquatic environment through various human activities (Forstner and Wittman, 1981). The environment works as a reservoir for all these pollutants and organic substances. In addition, sediments have higher capacity in reserving pollutants than that by water (Yen Nhi et al. 2013). Living organisms are considered biological dumpsters of pollutants and heavy metals, and therefore, they can be used to detect the changes in the concentration of heavy metals.

El-Metwally et al. (2019) recorded high levels of Fe, Pb, Zn, and Cu concentrations in sediments owing to different domestic and industrial sewage waste effluents. These metals precipitate into lower sediments through adsorption onto particulate matter (Salomons and Förstner, 1984) and accumulate to high concentrations, as recorded for Pb, Zn, and Cu in the sediment samples. Mansour et al. (2013) has revealed high concentrations (Cr, Fe, Mn, Zn, Pb) in sediments due to nonmonitored tourism-related activities such as landfilling operations and mooring boats.

3.2. Estimation of parasitic infection in fish

Fish are considered an important component of the ecosystem that must be monitored, as they hold a high place in the aquatic food chain and reflect the effect of harmful environmental changes. Fish are considered important biomarkers to assess biological pollution and have been used for studying bioaccumulation (Hellawell, 1986).

Table (1): Concentrations of heavy metals in µg /l from the three samples collected from three random locations from the seawater of the coastal area of Rabigh with the range and mean ± Standard deviation (SD). The Certified Reference Seawater Probe CASS-5 of the Institute of Environmental Chemistry, Canada, were used as quality control samples in µg /l.

Names of heavy metals detected at water surface	Zinc (Zn) ±	Iron (Fe) ±	Lead (Pb) ±
1	71.02	13.99	12.08
2	82.36	15.3	12.66
3	89.62	16.01	13.06
Range	71.02-89.62	13.99- 16.01	12.08- 13.06
Mean ± SD	81 ± 9.37	15.1 ± 1.02	12.6 ± 0.49
Reference CASS-5	0.72	1.44	0.011

Table (2): Composition of parasite species and their prevalence, abundance and intensity of *Carangoides bajad* captured from the coast of Rabigh, Saudi Arabia.

Species of parasites	No. of Examined fishes	No. of Infected fishes	Sex of fish (M/F)	Length (cm) of fish in mean ± SD	Weight (kg) of fish in mean ± SD	Total Number of parasite	Prevalence	Intensity	Abundance
***Hatschekia* (Poche, 1902)**	50	8	6 M / 2 F	34.71 ± 5.94	0.74 ± 0.23	16	16	2	0.32
***Bucephalus margaritae* (Ozaki & Ishibashi, 1934)**		22	6 M / 16 F	34.86 ± 4.58	0.56 ± 0.13	96	44	4.36	1.92
***Plagioporus ira* (Yamaguti, 1940)**		22	6 M / 16 F	37.45 ± 6.08	0.76 ± 0.26	68	44	3.09	1.36
***Tergestia bengalensis* (Gupta & Singh, 1985)**		6	6 M / 0 F	33.83 ± 3.87	0.55 ± 0.05	14	12	2.33	0.28
***Anisakis Physeteris* (Baylis, 1923)**		14	0 M/ 14 F	33.5 ± 4.99	0.54 ± 0.14	176	28	12.57	3.52
Total		40	18 M / 22 F	-	-	144	-	-	-

In this study, the relationship between water quality, parasites, and fish were assessed while considering the parasitic biodiversity that reflects the biodiversity and prevalence rate in the host. We identified the species of ectoparasites and endoparasites in 50 *C. bajad* fish. Forty were found to be infected with different species of ectoparasites and endoparasites (Fig. 3-7). Table (2) summarizes the comparison between the different parasites and their prevalence, intensity, and abundance in the examined fish.

The present study assessed the responses of ectoparasites and endoparasites to heavy metals in fish exposed to several parasites, and it reflected the environmental condition of the habitats that the host fish live in. Parasitic levels were high at less polluted sites (Nachev and Sures, 2009; Chapman et al. 2015). However, the infection and abundance of some parasites showed a clear relationship with increase in pollution. Meanwhile, the current study showed a diversity of endoparasites with a high abundance of *B. margaritae* in the infected fish. Although we found only one species of ectoparasites and less abundance in infected fish, this may have been caused by the direct effect of heavy metal pollution or indirect effect through metabolism or the immune state of the host (Kennedy, 1997). The prevalence of parasites, aggregation of parasites in the environment, difference in their numbers, and diversity in terms of place and time assures that parasites are a highly sensitive biomarker for heavy metals in the aquatic system.

Gheorghiu et al. (2007) mentioned that exposing guppy fish to Zn reduced the reproduction and survival of the ectoparasite *Gyrodactylus turnbulli*. Further, *G. turnbulli* was observed to be present on guppy fish in low to moderate concentrations; however, it decreased with higher concentrations of Zn, which affects the relationship dynamics between the parasite and the host. Increasing number of studies are being performed on the effect of heavy metal concentrations in both fish and their parasites (Sures et al. 2001), thereby indicating the role of heavy metals as pollutants that effect the ecosystem. Most such studies focused on the environmental stress and its role in changing parasite communities (Halmetoja et al. 2000). Pollutants can also affect the life stages of free-living parasites that can lead to a reduction in their population (Pietrock and Marcogliese, 2003). Given that there are different groups of parasites, they are transported along the food chain at different stages of their life. The changes in the formation and diversity of the heteroxenic parasitic communities can provide information on their environmental effect on the food web, which destroyed or enhanced the transformation of different parasites in that community (Marcogliese, 2004; Costanza and Mageau, 1999; Marcogliese, 2005).

Owing to the direct relation between heteroxenic parasites and the hosting community that lives freely on different nutrition levels, these organisms are considered sensitive biological factors to determine the health of an aquatic ecosystem (Marcogliese, 2004; Costanza and Mageau, 1999; Marcogliese, 2005). Amiard-Triquet et al. (2015) confirmed that changes in living organisms as a response to the harmful environmental changes is mostly caused by the effect of the pollutants and the disruption of the habitats. Parasites are considered an impact indicator where studies focus on the direct effects of pollutants on the viability and longevity of free-swimming stages (cercariae) and on the component changes of the populations and communities, as parasites are integrative parts of the food web inside the ecosystem. Environmental changes can be detected using parasites; if one of their developmental stages changes or if their hosts are negatively affected, several changes can occur in biodiversity patterns and markers associated with the parasite, which can be used as a measure of biodiversity. Therefore, we can predict and measure these population changes inside the parasitic community depending on the type and volume of human effects. Various studies, for example, have shown that toxic pollution reduces heteroxenic diversity, whereas parasites with direct live stages (monoxenic) are less effected. Further, the prevalence of some monoxenic parasites are probably caused by a weak immunity response of the host in a polluted environment (Marcogliese, 2004; Costanza and Mageau, 1999; Marcogliese, 2005).

3.3. Histological results

3.3.1. Gills

The gills and guts of the fish are major pathways for pollutants to enter the internal organs such as the liver and kidney via the blood (Burger et al. 2002).

The histopathological study of the gills revealed damages with different severities as gill tissues are tender sensitive tissues in the fish body. Further, gills are more exposed to pollutants or irritants in the water, which can destroy the composition of the gills. In addition, the gills are a preferred site for different parasites (Totoiu and Patriche et al. 2018) (e.g., copepods), and they damage the gills by feeding on the tender tissues and gill lamellae or on the blood within the blood lamellae, which cause losses in the surface area of the respiratory system (Lester and Hayward, 2006). Thus, gills are used in many studies as a strong and sensitive scale to assess the effect of pollutants on the cells and tissues of the body (Velmurugan et al. 2009). Gills in fish have complex compositions and functions, and therefore, studying gill tissues provides a clear image regarding the range of pollutants affecting the different types of cells that participate in many vital functions such as respiration, ionic exchange, and mucus secretion. Chavan and Mule, (2014) has recorded several histopathological changes in gills caused by fish being exposed to pollution by heavy metals. Gill filament damage to the gill tissue was observed in the studied fish in the form of elongation and twisting in the secondary gill lamella. This was caused by elongation of the lamella blood sinus attached to pillar cells and abnormal proliferation of chloride cells in the areas between the secondary lamella, which caused the swelling of the gaps between the filaments and detachment of the respiratory epithelia at the bases of the secondary gill lamella owing to leakage of fluids between the internal tissues of the gill lamella, known as edema (Supplementary plate 1). An increase in the size and numbers of chloride cells and their spread in the secondary lamella between the blood sinuses that was supported by pillar cells and separated respiratory epithelia was also noticed (Supplementary plate 2).

Further, the presence of gill filaments damage and an increase in the mucosal excretion in the parasitic infected places was observed in some fish gills because many parasites were found between the gill filaments and on the outer edge of the primary filaments due to eosinophilic infiltration (Supplementary plate 3). Lester & Hayward, (2006) mentioned various damages in the gills, including severe hemorrhage, inflammation, and exsanguination associated with the attachment of parasites. Endoparasites can be found in almost all internal organs of fish although the different types of parasites would have different specific fields inside the host (Feist and Longshaw, 2008). Usually, these parasites have complicated life cycles that depend on at least two hosts.

Lamellar swelling and fusion on both sides of various gill filaments were observed besides the previously mentioned tissue damages (Supplementary plate 4). Many histopathological changes in the gills were attributed to the fish being exposed to different pollutants with heavy metals; for example, dilation and congestion in the blood vessel of primary gill filaments, hyperplasia of epithelial cells between the secondary lamellae, which led to fusion and separation from the pillar system, vacuolation and necrosis of lamellar epithelial cells, congestion of central lamellar vein, and hyperplasia of lamellar epithelial cells (Chavan and Mule, 2014).

Atrophy of the gill filaments and blood congestion inside the main channel of the gill filaments has been observed, along with end elongation (Supplementary plate 5). Dogiel et al. (1961) found blockages in the blood vessels of the gill filaments, which lead to the atrophy of gill tips.

A degradation in some parts of the gill tissues were noticed, and the gills were separated by a pale color where complete degradation of the lamella blood sinuses was observed; this indicates the severity of the tissues damage. Complete sloughing of the fused respiratory epithelia in addition to lymphocytic infiltration and fibrosis of the main blood channel was also observed (Supplementary plate 6). This can be attributed to its infection with the Copepoda parasite that grips into the skin with its clawed antennae causing open wounds, which may promote bacterial infections. Ojha and Hughes, (2001) stated that one *Ergasilus bengalensis* parasite found on the gill filament of *Wallago attu* fish caused a 30% reduction in laminar flow, resulting in a 68% reduction in oxygen uptake (Lester and Hayward, 2006). Studies have shown that the presence of sea lice can be enough to cause stress to the fish infected (Ho, 2000). Further, when there is a high level of infection, sea lice can cause skin lesions and large open wounds (Lester and Hayward, 2006), which form a pathway for secondary bacterial infections (Egidius, 1985). Furthermore, parasitic copepods may serve as pathways for viral and bacterial diseases of fish; similarly, *Lepeophtheirus salmonis* may function as a vector for *Aeromonas salmonicida* as mentioned by Nylund et al. (1993). Although *C. bajad* is infected with small numbers of parasites that are not enough to result in mortality, it can affect their weight (Lester and Hayward, 2006).

The histological changes in the gills reveal an increase in the damage level of the tissues with an increase in the levels of pollution in seawater caused by industrial and domestic waste that accumulate large quantities of heavy metals such as Cr, Ni, Pb, Cu, Cd, and Zn (Manzoor et al. 2006).

3.3.2. Intestines

Severe tissue damages in the intestines caused the complete decomposition of the mucosal layer, lymphocytic infiltration of all intestinal layers, and the presence of parasitic protozoa in the sample fish. This can be attributed to the direct contact of heavy metals with the intestines through food and water and with foreign bodies, and with environmental pollutants such as xenobiotics that fish ingest (Lemaire et al. 1992). Sharma et al. (2014) mentioned that exposure to heavy metals can damage mucus tissues and the intestinal tract and the skeletal, central nervous, and reproductive systems.

Further, the histological examination of the fish revealed asymmetry in the shape and size of intestinal villi (heterogeneity) of some fish. Villi in some plates appeared swollen and some other degenerated with complete sloughing of mucosal membranes; further, a disorder in the general organization of the villi and detachment of the mucosal layer from the submucosal and the separation of the muscular bundles were observed in some villi (Supplementary plate 7). Similar results were recorded by Samanta et al. (2016), where she found the fusion of stomach microvilli, pigmentation in different parts of the microvilli, appearance of vacuolation, cell swelling, aggregation of blood cells, and hemorrhaging after endosulfan exposure in the stomach of *C. punctatus.*

These results are consistent with the results of Prado Rodrigues et al. (2018) who noted goblet cells, the thickness of intestinal villi, blood cell infiltrate, fusion of the villi and eosinophils in the intestinal villi of *Danio rerio* fish exposed to manganese.

A study by Maurya et al. (2019) observed many histological damages in the intestines, such as edema, inflammation, atrophy, necrosis, hemorrhage, degenerated muscle layer, degenerated mucosa, and necrotic debris when fish were exposed industrial wastewater. In addition, mutations of the epithelial mucosa were observed between the villi where the cells were pressurized and carried various long microvilli (Supplementary plate 8).

The presence of various parasitic worms has been observed in the intestinal cavity of some fish. These intestinal plates were distinguished by the sloughing of intestinal mucosa and the intestinal cavity being filled with hemorrhage and inflammatory cellular infiltration and dead tissues (Supplementary plate 9); this and that can be due to infection with various endoparasites (*B. margaritae*, *P. ira, T. bengalensis*, and *A. physeteris*). Marchiori et al. (2010) found *B. margaritae* adult forms in the intestine and pyloric cecum of *Menticirrhus americanus* in their study. In a previous study, it was mentioned that acidic cells are activated by parasitism as their numbers increase in inflammation areas (Patt and Patt, 1969). Amal, (1992) mentioned that the mature worms of *Anisakis* are found in the digestive channel and often in the blood system and reproductive organs. Yoshinaga et al. (1989) mentioned that all internal parasites have severe effects on the fish that it infects.

Severe deformation in the villi and intestinal cavity filled with parasites, dead tissues, and bloody hemorrhage (Supplementary plate 10) were observed in parasitic infected fish.

The tissue plates of the fish intestines were distinguished by distress in the organizational composition of the intestinal tissues in various fish, and there was difficulty in differentiating the intestinal villi that sloughed and detached inside the intestines or appeared as deformed in a series of fibrous ridges (Supplementary plate 11). Woodward et al. (1995) found damages in the intestinal tissues in the shape of vacuolar degeneration, sloughing of the mucosal epithelia, and a shortage in the zymogen numbers in *Salmo trutta* that feeds on invertebrates infected with heavy metals.

A decrease in the cellular content and fibrosis of the blood vessels was observed in the submucosal layer, which indicates a clear shortage in the blood supply of the intestinal tissue or the occurrence of lymphocytic infiltration. Although the muscular layer is clearly distinguished, a shortage is observed in the muscular bundles in addition to their inability to be defined into layers and their detachment from the submucosal layer. Many parasites were defined between the dead tissues in the cavity of some intestines.

3.3.3. Liver

Tissue analysis of the liver by using a light microscope revealed that based on the toxicity caused by pollutants, the histopathological results change. They appear in the form of disturbances in the natural compositions of the liver, where a disorganization in the hepatic parenchyma is noted, along with fatty infiltration and vacuolar degeneration in the liver tissue. The nucleus was pushed to the edge in many cells. Vacuolar degeneration was distinguished by a severe shortage of the cytoplasmic content and an aggregation of cytoplasmic granules near the nucleus. Necrosis in the nucleus of some hepatic cells appeared along with decomposition and karyolysis of the nucleus (Supplementary plate 12), in addition to dilation and sinusoidal congestion. A study by Mekkawy et al. (2012) on the liver of *Oreochromis niloticus* exposed to lead nitrate displayed an increase in the amount of hepatocellular lipid deposits. This was further confirmed by Joseph & Asha Raj, (2018) when they studied the liver of *Anabas testudineus* in a site polluted with Pb, Ni, Zn, As, and Cd and they noticed a distortion of the hepatic cells and necrosis; they also pointed out that necrosis is a symptom of cell death. Maurya et al. (2019) found that damages in the fish liver, intestine, and heart tissues exhibited dilated sinusoids, hemorrhage, enlarged hepatocytes, vacuolar degeneration, degenerated hepatic cells, karyolysis, pyknotic nuclei, and necrosis which are attributed to exposure to pesticides.

Bile duct proliferation and vacuolar degeneration were observed in the epithelial cells as they are in the hepatic tissues of the fish; in addition, the presence of parasitic worms and the degradation of its epithelial tissues were also observed. Circulatory disturbances formed in the endocrine glands of the fish, and their congestion and repletion with stagnant blood cells were accompanied by the aggregation of macrophages around the vessels (Supplementary plate 13). A study by Akhter and Saha, (2013) on the liver of *Channa punctatus* fish observed fatty infiltration in the hepatic cells, vacuolar degeneration, parenchymal vacuolation, focal areas of necrosis, dilation and thrombosis formation in central vein, dilation and congestion in blood sinusoids, blood vessels inside the hepatic tissue, hydropic degenerations, necrosis, and fibrosis due to exposure to Fenitrothion. Similarly, Chavan & Mule, (2014) found dilation in the blood vessels, hemolysis caused by the destruction of erythrocytes, inflammation of hepatic cells, eccentric nuclei, vacuole appearance, and congestion in blood sinusoids, which appeared in the liver tissue after lead exposure. Cytoplasmic vacuolation, intravascular hemolysis in blood vessels, dilation, and congestion in sinusoids and venules and cellular degeneration, and focal necrosis were also observed in the liver of *Cirrhinus mrigala* fish.

In some liver plates of the fish (Supplementary plate 14), granulomata inflammation, which forms because of caseous necrosis of the hepatic tissues inside was observed. This is surrounded by inflammatory cellular invasion of white cells and surrounded externally with fibroblasts that excrete a layer of collagen fibers, and thus, it is called encapsulated granuloma. Further, severe lymphocytic infiltration was observed in hepatic cells surrounded with inflammatory granules. Various studies found fish with granuloma formation and they were characterized with caseous matter within the layers of epithelium cells and fibrous tissue owing to the exposure to different pollutants (Bin-Dohaish et al. 2004; Burkitt et al. 1996).

From the morphological investigation, the inflation of the liver was observed in other fish, as the ability of the tissues to be pigmented decreased and it became pale. Ballooning degeneration was observed in hepatic cells, in addition to a severe decrease in its cytoplasmic content, adhesion of its plasmatic membranes, pyknosis in some cells and karyolysis in others, and swelling in many cells (Supplementary plate 15). Sinusoidal collapse was noticed owing to the swelling of the cells and an increase in the inflammatory lymphocytic infiltration from the lymphatic cells. A histopathological study by Velmurugan et al. (2009) on the liver of *C. mrigala* fish when exposed to dichlorvos with different concentrations found histological changes of cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids, and nuclear hypertrophy.

In some liver plates, parasitic worms were present in the liver tissues with the tissues surrounding the infection (Supplementary plate 16). Further, hepatic tissue degeneration was caused by infection with parasites and inflammatory cellular invasion (Supplementary plate 17). Suzuki, (1982) mentioned that the larvae of nematodes were found in 29 species of fish and 2 species of squids; these larvae were found freely in the digestive cavity (stomach, intestines) or encysted in the abdominal cavity and above different organs of the internal gut. The Red Sea coast of Jeddah is considered a habitat for the geographical spreading of the larvae from two species of *Anisakis simplex* and *A. physeteris* (Bakhraibah, 1999).

Some studies proved that the necrosis and vacuolar degeneration of the liver tissues was caused by viral, parasitic, and bacterial infections when infected with contaminants. Pollutants such as heavy crude oil caused a decrease in the glycogen levels of the liver and changes in the fats of the liver (Carls et al. 1998).

4. CONCLUSION

From this study, we concluded that parasites are not only organisms that threaten the life of their hosts but also highly responsive living creatures. In addition, they are a strong biological tool to understand and determine ecological changes by representing the sensitivity of the organs to environmental changes and focusing on the physiological and histological changes that parasites can have on their host. Parasitic communities reflect the natural structure of the host habitat. We suggest that parasites are good biomarkers to prove the existence of environmental pollutants, assess the health of the ecosystem, and to continue the research on environmental pollutants introduced by human activities.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

EJBD, AOB designed and performed the experiments and also wrote the manuscript. EJBD, AOB performed the parastic and histological examination and data analysis. EJBD reviewed the manuscript. All authors read and approved the final version.

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