Microbial pollution in inland recreational freshwaters of Quetta, Pakistan: an initial report

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ABSTRACT

Parasitic contamination of surface waters especially recreational waters is a serious problem for under-developed nations like Pakistan, where numerous outbreaks of parasitic diseases are reported each year. In the current study, parasitic presence in two surface waters (Hanna Lake and Wali-Tangi Dam) of Quetta was monitored quarterly for 1 year. The methodology involved the pre-concentration of the water samples and the subsequent preparation for the microscopic search of parasites. Physico-chemical and bacteriological variables were also studied. Wet staining, modified Trichrome staining, and modified acid-fast staining methods were used to identify various parasitic forms (cysts, oocysts, eggs, trophozoites). Collectively 11 parasitic elements (10 in Lake and 8 in Dam) belonging to 10 species were recorded, many of which are potential human pathogens. The species identified include Trichomonas sp., Isospora sp., Balantidium coli, Cryptosporidium sp., Entamoeba spp., Amoebas, Microsporidium sp., Endolimax nana, Ascaris lumbricoides, and Giardia spp. Parasitic contamination remained persistent in both locations throughout the year independent of physico-chemical parameters (temperature, EC, pH, turbidity, and DO) and bacterial concentration of water. Reliance on bacterial presence for monitoring of recreational waters can be a risk for tourists. Entamoeba spp. and A. lumbricoides may be used for surface water monitoring in these waters.

Key words: Ascaris, Cryptosporidium, Entamoeba, Giardia, parasitic pollution, recreational waters

HIGHLIGHTS

• Initial report on parasitic pollution in surface recreational waters in Pakistan.
• Parasitic pollution is highly persistent in both recreational waters.
• Reliance on bacterial parameters only for recreational waters can be risky.
• Entamoeba and Ascaris lumbricoides are potential candidate species for monitoring.
INTRODUCTION

Recreational waters are those surface waters that are used for entertainment purposes by people. In general, recreational waters include waters of swimming pools, hydro-massage baths, hot springs and natural surface waters (rivers, lakes, ponds, etc.), and marine coastal waters (Mavridou et al. 2018; Valeriani et al. 2018). These natural aquatic environments are an alternative for the population that lacks the financial resources to access private pools, spas, and manmade environments. Since the last two decades, researchers have linked the exposure to recreational waters with public health issues (Harrison 2018; Leoni et al. 2018). About 11% of the worldwide water-borne outbreaks are attributed to parasites (Balduresson & Karanis 2011; Yang et al. 2012). Approximately 1.7 billion diarrhoeal cases are associated with protozoan parasites in the world, and the diarrhoea-associated deaths are the second leading cause of mortalities in children (Efstratiou et al. 2017; Omarova et al. 2018; Jain et al. 2019). In the USA, 80% of the diseases acquired in recreational settings are due to infections produced by microorganisms, mainly parasites, and bacteria (Yoder et al. 2012). The most common health issues reported to be linked with aqueous recreational settings are intestinal, respiratory, eye, ear, and skin infections. The major transmission pathway is the oral route; however, a pathogen may enter the body via ears, eyes, nose, or damaged or rashed skin (Pitlik et al. 1987). Pollution sources for recreational waters include but are not limited to direct faecal pollution of humans and animals, storm-water runoff, pollution from boating activities, and agricultural runoff (EPA 2021). In the USA, 43% of outbreaks reported during 2005–6 were from recreational settings, and two of them were parasitic infections (Yoder et al. 2008). Among them, Cryptosporidium and Giardia were responsible for most illnesses from recreational water resources (Yoder et al. 2012; Adeyemo et al. 2019). Both the parasites persist in the environment for longer periods than certain bacterial pathogens, are more resistant to disinfection treatments, and have low infective doses (Adeyemo et al. 2019). Another important aquatic parasitic group is free-living Amoebas. Unlike other human pathogenic parasites, they have the ability not only to survive in an aqueous medium but also to reproduce, sporadically invade a host, and live as parasites within their tissues (Certad et al. 2017). On the other hand, in developing countries, diseases caused by parasites are more common owing to poor sanitation issues and climatic conditions that favour the proliferation of parasites.

In Pakistan, the most common water-related parasitic diseases include cholera, typhoid, Giardiasis, dysentery, intestinal worms, diarrhoea, and Cryptosporidiosis. These diseases comprise 80% of the illnesses in the country and are responsible for 33% of all deaths (Daud et al. 2017). In Asia, the highest ratio of infant mortality (60%) due to water-related diarrhoea exists in Pakistan (Daud et al. 2017). The most common water-borne diseases reported from Quetta, Pakistan include typhoid, hepatitis, gastroenteritis, and dysentery (Khattak 2011). The role of exposure to recreational waters of Hanna Lake and Wali-Tangi Dam may have a contribution to the burden of water-borne diseases in Quetta. The quality of recreational waters in Quetta needs to be investigated to better understand the dynamics of water-borne diseases in the city. Recreational water
standards and monitoring are not implemented currently in Pakistan. The self or voluntary monitoring of recreational waters (swimming pools in hotels) where adopted are only confined to bacteriological tests. Although some studies suggest that bacterial parameters are not suitable to predict the occurrence of parasites in water (Wen et al. 2020); however, the use of only bacterial parameters to declare the waters safe is a common practice in the world (Pitlik et al. 1987). Several reports have shown that parasites can be used as potential indicators of contamination in waters (Slodkowicz et al. 2015).

Several studies conducted globally on the parasitological quality of water samples have detected many parasitic species (Daud et al. 2017; Leoni et al. 2018; Valeriani et al. 2018). Minute quantities of parasites in water are sufficient to produce a disease, but such quantities cannot be detected without the prior concentration of a large volume of water (Khattak 2011; Poma et al. 2012, 2013). An ultrafiltration membrane is used to retain and recover the pathogens from the large volume of water for later detection by special techniques (Poma et al. 2013). The objective of the current study was to detect the possible presence of parasitic elements in recreational aqueous environments of Hanna Lake and Wali-Tangi Dam in Quetta, Balochistan, Pakistan and to compare its presence with bacterial indicators.

METHODS

This study has been conducted for Hanna Lake and Wali-Tangi Dam, which are located east of the city of Quetta. Hanna Lake is about 5 km from Quetta at 30.254311N, 67.099476E, while Wali-Tangi Dam is about 19 km from the city at 30.26432N, 67.24625E (Figure 1).

Figure 1 | Location map and sampling sites of Hanna Lake and Wali-Tangi Dam (google earth).
A quarterly sampling of 24 L of water in clean plastic buckets (rinsed with same water) was done from Hanna Lake and Wali-Tangi Dam during 2014–5 (April 2014-spring, July 2014-summer, October 2014-autumn, and January 2015-winter). Total four water samples (one in each season) were collected, 15–20 cm below the water surface and well above the bottom sediments to avoid any floating and settled materials. The bucket was covered with the bucket cap and moved to the laboratory within an hour for processing. Quarterly sampling was also carried out for bacteriological tests following the Standard Method for the examination of surface waters (Eaton et al. 2005). Samples were collected in sterile bottles of 500 ml size, put into ice-cubes containing containers, and moved to the laboratory for analysis. Samples were processed within 4 h of sampling. Common physico-chemical variables including pH, electrical conductivity (EC), turbidity, dissolved oxygen (DO), and temperature (°C) were determined at the spot using Hanna pH meter, Hanna conductivity meter, Portable Hanna turbidity meter, X-Tech portable DO meter, and glass thermometer, respectively.

Total coliform bacteria (TC) and faecal coliform (FC) were counted using the multiple tube method in MacConkey broth, incubated at 37 ± 0.5 and 44 ± 0.5 °C, respectively, for 24 h (Eaton et al. 2005). The bacterial numbers of the samples were estimated at the most probable number in 100 ml (MPN/100 ml). TC and FC were confirmed in the MPN method using the US FDA protocol (Hitchins et al. 1998). For Escherichia coli and enterococci, the membrane filtration technique was used: E. coli (ECL), on modified m-TEC agar (Fluka USA) at 44.5 °C for 24 h (EPA 2002a), and enterococci (EN), on mE agar (Difco-USA) at 41 °C for 48 h and confirmation on esculin iron-agar (EIA) at 41 °C for 20 min (EPA 2002b). The results were expressed in CFU/100 ml.

For parasitological determination, samples were concentrated by ultrafiltration using two sequential hollow fibre modules (Rajal et al. 2007). Twenty litres of water was fed into the feed tank system and pumped through an ultrafiltration unit. The retained volume was re-circulated through the ultrafiltration unit several times until it reaches the lowest possible retained volume of 40–60 ml. The filtrate was discarded. To recover any adsorbed particles on the membrane, two elution steps were performed for 15 min each with 15 ml elution solution (0.05 M for glycine/NaOH and 0.1% Tween 80) and without permeate (Poma et al. 2013). The eluates were combined with the retained volume to form the concentrate for parasitic analysis.

Before the parasitological determination, 20 ml of concentrated water sample was passed through three layers of gauze to remove debris and particles which could be mistaken for parasitic elements. Each sample was divided into two and each of them was concentrated again by applying two methods, the float sucrose method (Sheather 1923) and the sedimentation method by centrifuge (Magaro 2013). The pellet was centrifuged at 1,000 rpm for 5 min and subsequently re-suspended in 10% sucrose solution. To avoid loss of the characteristic morphology of parasitic elements, samples were preserved in three different ways, i.e. 10% formaldehyde, in sodium-acetate-formalin (Yang & Scholten 1977), and in merthiolate-iodine-formaldehyde (Sapero & Lawless 1953).

Identification of trophozoites, cysts, oocysts, eggs, and larvae was performed by direct microscopy of the preserved samples. Wet samples with Lugol’s solution were prepared to identify trophozoites or cysts of protozoa and eggs or larvae of helminths. Wet stains with eosin or methylene blue were used to identify oocysts, with modified acid-fast stain for Cryptosporidium oocyst. Permanent trichrome-stained smears of the concentrated original samples were prepared to detect spores of protozoa and microsporidia. The count of helminth eggs was performed by the method of quantification of Stoll (Stoll & Hausheer 1926).

**RESULTS AND DISCUSSION**

Total 11 parasitic elements were identified in both Hanna Lake and Wali-Tangi Dam, 10 in the lake and 8 in the dam (Table 1, Figure 2). These include *Trichomonas* sp. (trophozoite), *Isospora* sp. (oocyst), *Balantidium coli* (cyst), *Cryptosporidium* sp. (oocyst), *Entamoeba* spp. (trophozoite), *Entamoeba* spp. (cyst), *Amoebas* (trophozoite), *Microsporidium* sp. (spore), *Endolimax nana* (cyst), *Ascaris lumbricoides* (egg), and *Giardia* spp. (cyst). To survive, these parasitic species must have certain characteristics which make them successful to live outside the body of the host in an aqueous environment. Such properties include their ability to transmit through water, high infectivity rate, proliferate in an aqueous medium, and resistance when outside the body (Wilkes et al. 2009). In the current study, parasites are detected in latent forms like cysts, oocysts, and eggs. Parasites are excreted as these latent forms with faeces into the environment. Although they cannot multiply in this form outside a body but can pass through it as living forms until they infect a host. Opportunistic pathogens like amoeba are also
Table 1 | Parasitic element (Number/Litre) in the Hanna Lake and Wali-Tangi Dam, Quetta, Balochistan

| #  | Parasites (form)       | Hanna Lake |          |          |          | Wall-Tangi Dam |          |          |          |          |          | Total |
|----|------------------------|-----------|----------|----------|----------|---------------|----------|----------|----------|----------|----------|--------|
| 1  | Trichomonas sp. (trophozoite) | –         | –        | –        | –        | –             | 2        | 4        | –        | –        | 2        | 6      |
| 2  | Isospora sp. (oocyst)    | –         | –        | –        | 4        | –             | –        | –        | –        | –        | 2        | 4      |
| 3  | Balantidium coli (cyst) | 4         | 8        | 8        | 10       | 9             | 7        | 9        | 9        | 9        | 71       |        |
| 4  | Cryptosporidium sp. (oocyst) | –         | –        | 2        | 3        | –             | –        | –        | –        | –        | 5        |        |
| 5  | Entamoeba spp. (trophozoite) | –         | 6        | 5        | –        | 2             | 4        | 5        | –        | –        | 22       |        |
| 6  | Entamoeba spp. (cyst)    | 11        | 8        | 8        | 10       | 9             | 7        | 9        | 9        | 9        | 71       |        |
| 7  | Amoebas (trophozoite)    | 6         | 9        | 7        | 5        | 7             | 5        | 2        | 6        | 4        | 47       |        |
| 8  | Microsporidium sp. (spore) | 10        | 9        | 17       | 14       | 11            | 9        | 19       | 18       | 18       | 107      |        |
| 9  | Endolimax nana (cyst)    | –         | 5        | 8        | –        | –             | 6        | 4        | –        | –        | 23       |        |
| 10 | Ascaris lumbricoides (egg) | 6         | 8        | –        | –        | 6             | 7        | –        | –        | –        | 27       |        |
| 11 | Giardia spp. (cyst)      | –         | 5        | 4        | –        | –             | 6        | 3        | –        | –        | 18       |        |
|    | Total (type/count)       | 4/33      | 6/39     | 7/45     | 7/33     | 4/33          | 7/34     | 7/40     | 5/36     |          |          |        |

detected in the current study, which remains free-living in water and can enter human or animal bodies as parasites if chance exists (Attariani et al. 2020; Beknazarova et al. 2020).

Identification of parasites

Trophozoites of Trichomonas were identified based on morphological characteristics under the microscope (Soulsby 1968). They were recognized from the anterior single nucleus, anterior flagella, and characteristic shape. The posterior flagellum and undulating membrane were not apparent and could have been damaged during sample preparation. The trophozoite was 10 μm in width and 15 μm in length. Lugol’s iodine staining was used to identify trophozoites of Trichomonas. The morphological features observed under the microscope were adequate to confirm the genus Trichomonas; however, owing to the blurriness of some features, the species-level identification was not possible. Possibly these can be avian Trichomonas rather than human pathogens.

All the four oocysts of Isospora sp. detected during the current study were mature oocysts with clearly divided protoplasm called sporocysts (Figure 2(h)). The Isospora oocyst was identified based on the presence of two apparent sporocysts and a thick oocyst wall. Sporozoites were faintly visible within each sporocyst. The size of the oocysts length recorded during the current study was 32 μm. It was observed in wet preparation with eosin stain and acid-fast staining. Sporocysts were stained deep red with acid-fast staining. The oocyst was seen and identified under 100× and 400× magnification of the microscope. B. coli cysts identified during the current study were about 50–60 μm in size (Figure 2(a)). They were round to slightly oval with apparent macronucleus. The double wall was slightly distinguishable, while no cilia were seen on any of the four cysts identified. Contractile vacuoles were observed in a couple of cysts. The cyst was appearing green with greenish background with Trichrome staining. They were observed in 40× and 100× magnifications under the microscope.

Cryptosporidium oocysts appeared as bright red spherical balls with a green background using modified acid-fast stain, and with iodine stain, they appeared colourless. They were very small ranging from 4 to 7 μm in size (Figure 2(g)). Sporocysts were also observed in one of the Cryptosporidium oocysts. They were observed and identified in 1,000× magnification. Entamoeba sp. cysts and trophozoites were identified based on the morphological features under the microscope described in the literature (Soulsby 1968; Shahnazi & Jafari 2010; Mohamed et al. 2016; Bekele & Shumbej 2019). Entamoeba sp. cysts were identified during the current study based on their quadrinucleate structure and smaller size (Figure 2(e)). Entamoeba cysts recorded were about 10–15 μm in size and spherical with typical four nuclei. Lugol’s iodine stain was used to stain the cyst. Entamoeba trophozoite identified during the current study was elongated and oval in shape with a single conspicuous globular nucleus in Lugol’s iodine stain (Figure 2(f)). It was 20 μm in length and 10 μm in width. Detection of Entamoeba trophozoite in the current study is parallel to findings of earlier studies that have detected the fragile trophozoite parasitic forms despite concentration and preparation steps (Ayaz et al. 2011; Hatam et al. 2015).
Microsporidium spores detected were very small in size with 2 μm in length. They appeared pinkish in modified Trichrome staining (weber staining) and were only visible under 1,000× magnification. The spores were identified based on morphological features described by Garcia (2002). A horizontal band (polar tube) was evident in the Microsporidium spores identified during the current study. *E. nana* cysts were identified based on the morphological characteristics detailed by Soulsby (1968). Oval to spherical-shaped cysts of *E. nana* with four nuclei were distinguished from other cysts by their extremely smaller size (5–6 μm) and were only visible in 1,000×. With iodine staining, cysts appeared pale with pale brown nuclei. *A. lumbricoides* eggs were identified in wet mount and iodine staining using guidelines for morphological characteristics described by Soulsby (1968) and Cuomo et al. (2009). Fertilized eggs were readily identified due to the thick external wall which is bumpy and brown without staining (Figure 2(b)). These were oval in shape with 60 μm in length and 40 μm in width. Unfertilized elongated eggs remained absent in the present study. *Giardia* cyst was identified based on morphological features under a microscope described by Soulsby (1968). Cyst detected in this study was oval in shape and 12 μm in length, with two nuclei and fibril. In iodine staining, it appeared light brown with light blue or white background.

*Microsporidium* spores detected were very small in size with 2 μm in length. They appeared pinkish in modified Trichrome staining (weber staining) and were only visible under 1,000× magnification. The spores were identified based on morphological features described by Garcia (2002). A horizontal band (polar tube) was evident in the Microsporidium spores identified during the current study. *E. nana* cysts were identified based on the morphological characteristics detailed by Soulsby (1968). Oval to spherical-shaped cysts of *E. nana* with four nuclei were distinguished from other cysts by their extremely smaller size (5–6 μm) and were only visible in 1,000×. With iodine staining, cysts appeared pale with pale brown nuclei. *A. lumbricoides* eggs were identified in wet mount and iodine staining using guidelines for morphological characteristics described by Soulsby (1968) and Cuomo et al. (2009). Fertilized eggs were readily identified due to the thick external wall which is bumpy and brown without staining (Figure 2(b)). These were oval in shape with 60 μm in length and 40 μm in width. Unfertilized elongated eggs remained absent in the present study. *Giardia* cyst was identified based on morphological features under a microscope described by Soulsby (1968). Cyst detected in this study was oval in shape and 12 μm in length, with two nuclei and fibril. In iodine staining, it appeared light brown with light blue or white background.

**Figure 2** | (a) *Balantium colli* cyst (Trichrome staining), (b) *Ascaris lumbricoides* egg (Lugol’s iodine), (c) *Cryptosporidium* oocyst (acid-fast staining), (d) *Endolimax nana* egg (iodine staining), (e) *Entamoeba* spp. cyst (Lugol’s iodine), (f) *Entamoeba* trophozoite (Lugol’s iodine), (g) *Giardia* spp. cyst (Lugol’s iodine), (h) *Isospora* oocyst (no staining), (i) *Trichomonas* trophozoite (Lugol’s iodine), (j) *Microsporidia* spores (modified Trichrome staining).
Biodiversity of parasites

The highest biodiversity with seven types of parasitic elements in Hana Lake was recorded in autumn and winter while similar numbers in Wali-Tangi Dam were recorded in summer and autumn (Figure 3), which shows the highest numbers of parasitic elements during autumn (following summer) in both water bodies. Minimum total count was observed in spring (following winter). The vast majority of the recognized parasitic elements in both the waters belong to protozoa, which are generally associated with disease transmission in water. In the current study, in both the aquatic environments monitored, amoebae were found generally in every sample. The presence of Amoebas in both environments and all seasons reflects its ability to withstand large temperature fluctuations of Quetta. While not all amoebas found in the environment (e.g. in water or soil) cause the disease, some of them can host bacteria, such as Legionella spp. and others (Edagawa et al. 2019), which may occasionally become pathogens in immunosuppressed populations (McMullen et al. 2017).

Similarly, Entamoeba is detected in all seasons in both Hanna Lake and Wali-Tangi Dam in cyst form, while its trophozoite form has not been recorded during spring in both the waters and summer in Hanna Lake. Although all the protozoans belonging to the Entamoeba group are not pathogenic for humans, however, some like Entamoeba histolytica are dangerous for humans. Intestinal amoebiasis disease caused by E. histolytica is the third most widespread parasitic disease responsible for more deaths in the world, after malaria and schistosomiasis (Tasawar et al. 2010). The presence of Entamoeba indicates possible faecal contamination of the aquatic environment. Furthermore, the eventual ingestion of this protozoan may be accompanied by ingestion of other faecal parasites that are pathogens. There is a thin line demarcation between commensalism and parasitism; hence, many times the parasites living as commensals in a host may become parasites occasionally. Prevalence of E. histolytica related illnesses is considerably high among parasitic diseases in Pakistan and around the world (Ahsan-ul-Wadood et al. 2005; Haider et al. 2012; Yakoob et al. 2012). Based on stool analysis, the rural prevalence of E. histolytica is reported to be 13.6–63.8% in the general public in Pakistan (Khan et al. 2019). Several genera of Entamoeba including E. dispar, E. moshkovskii, E. poleki, E. coli, and E. hartmanni may live as commensals in the human intestine, while E. histolytica causes intestinal and extra-intestinal diseases (Yakoob et al. 2012).

The pathogenic status of E. nana, which is a cosmopolitan amoeba, is doubtful. It is a commensal of the human intestine and some animals and is highly prevalent in tropical regions (Shah et al. 2012). Precarious living conditions and the consumption of nutrients from polluted water and food are some of the factors responsible for the presence of this parasite in the population (Mukhiya et al. 2012). In the current study, E. nana was detected in the summer and autumn in both Hanna Lake and Wali-Tangi Dam. Its presence has also been reported by other authors around the world in water, wastewater from a treatment plant, cooked food, stools of children, soil, faecal matter, and urine (Elele & Gboeloh 2017; Ribas et al.

Figure 3 | Seasonal changes in diversity and total count of parasites in Hanna Lake and Wali-Tangi Dam.
Many studies have associated the presence of *E. nana* with *Blastocystis hominis* (Alzate et al. 2020). Although both parasites are considered non-pathogenic, few studies have associated *E. nana* with diarrhea in children (Belkessa et al. 2021).

*Cryptosporidium* sp. was detected in the autumn and winter seasons in Hanna Lake waters. The mature oocyst of *Cryptosporidium* detected in the current study is the infecting form of the parasite, which increases its potential pathogenicity. This form is highly resistant to withstand adverse conditions in recreational water. It can withstand chlorination levels of 0.2–0.5 mg/l residual limit used in pools and treated waters. It has a low infectious dose of only 1–10 oocysts and immediately becomes infectious after excretion by the previous host (Kothary & Babu 2001). However, *Isospora* oocyst, which is found only in winter in Hanna Lake waters, needs external maturation before being infectious after ejection from the previous host.

Cysts of *Giardia* spp. were observed in both environments during the autumn and winter. This cosmopolitan genus is already well known for being the cause of water-borne diseases along with *Cryptosporidium* sp. These parasites are common in wastewater; hence, their presence is suspected to be related to domestic or industrial discharge (Bertrand et al. 2004). The ubiquitous presence of pathogens and inefficient effluent treatments lead to the proliferation of these organisms in surface waters, with a high probability of causing adverse effects not only in humans but also in animals.

*Trichomonas* sp. trophozoite was found in summer and autumn in Wali-Tangi Dam only (Figure 2(i)). Species-level identification was not possible with the current methodology. Identification of the fragile trophozoite form of *Trichomonas* sp., in the present study despite sample filtration steps, are parallel to several studies where the fragile form of parasites was recorded despite preparation steps (Ayaz et al. 2011; Hatam et al. 2015). This species possibly can be avian *Trichomonas*. Trophozoite of *Trichomonas* sp. (*T. vaginalis*) is reported to remain viable for up to 30 h in swimming pools despite residual chlorine (Pereira & Benchimol 2008).

*Microsporidium* is found in both the waters of Hanna Lake and Wali-Tangi Dam in all seasons and in large numbers. These are alarming signs for Quetta residents who rush to these spots frequently. *Microsporidium* is an insufficiently described genus and several of the species belonging to this group are human pathogens, hence, are considered as emerging water-borne pathogens. Many species of *Microsporidium* have been reported to be aetiological agents for diseases in humans, especially in immune-compromised individuals (Nooshadokht et al. 2017).

*B. coli* was found in only Hanna Lake in the summer. Water is the main vehicle for this ciliate which infects humans and animals and causes diarrhea and dysentery (Al-Musawi 2016). This organism is the largest among the protozoa and its trophozoite can reach up to 150 μm in length. However, in the current study, *B. coli* is only found in cyst form once during the whole year.

Only one metazoan species, *A. lumbricoides*, is recorded in the current investigation during spring and summer in both environments. It is a soil-transmitted metazoon and its eggs require soil for maturation and to become infective. This soil-borne pathogen causes ascariasis, an infection of the small intestine. It causes important social and health implications for school-aged children in Asia and Africa belonging to low socio-economic and endemic populations (Asaolu et al. 2002). It is prevalent in remote parts of Pakistan including Balochistan. In the current investigation, this pathogen is detected in egg form, which may hatch at a suitable time to produce worms. In Quetta, the temperature seems to limit this parasite to only summer seasons, an observation parallel to the studies conducted in other parts of the world (Soriano et al. 2001).

The rationale behind the confinement of *Cryptosporidium* sp. (oocyst), *Isospora* sp. (oocyst), *B. coli* (cyst) in the lake, and *Trichomonas* sp. (trophozoite) in the dam is not strong. The ecological conditions appear similar in both the waters, except for the quantum of human interaction. Human interaction with water is higher in Hanna Lake, where more people visit the lake compared to Wali-Tangi Dam. The presence of *Cryptosporidium* sp. And *B. coli* in the lake, which are human pathogens, may be attributed to greater human activities in lake water. The presence of *Isospora*, which is a known pathogen of canines, may be linked with the greater presence of dogs and cats at Lake compared to Dam. Confinement of *Trichomonas* sp. in only Dam is unique.

In the current study, three parasites, *Entamoeba* spp. (cyst), Amoebas (trophozoite), and *Microsporidium* sp. (spores), were recorded during all the seasons at both locations. *Microsporidium* sp. (spores) and *Entamoeba* spp. (cyst) were recorded in the highest numbers during all the seasons in both the waters. The presence of *Microsporidium* in all the seasons despite great fluctuations in the temperature of Quetta city may be attributed to its ability to infect a wide range of hosts and its ability to withstand temperature fluctuations. They can infect a variety of hosts, e.g. warm-blooded, cold-blooded, vertebrates, and invertebrates (crustaceans, aquatic insects, and others) (Abdel-Baki et al. 2009). Additionally, its ability to infect the host
remains mostly independent of the prevailing temperature of water (Nascimento et al. 2007). The presence of amoebic trophozoite during all the seasons may be attributed to its ability to withstand great temperature fluctuations, a great diversity of species, and a wide range of hosts. Water-borne parasite Entamoeba spp. has less variety of hosts compared to Microsporidium and Amoeba, and its ability to infect in various temperature regimes is also less known. Its presence throughout the season in both waters is unique. Spores and cysts are more resistant to adverse environmental conditions compared to trophozoite form, and their presence in larger numbers is normal.

**Seasonality of physico-chemical parameters and parasites**

Seasonal variation in physico-chemical parameters can influence the presence or absence of prokaryotes. Significant seasonal change in EC was not recorded in Hanna Lake and Wali-Tangi Dam (Figure 4). However, EC recorded in Wali-Tangi Dam was slightly higher than Hanna Lake. Major seasonal fluctuations in pH were not observed in both environments. In Hanna Lake, it ranged from 7.9 in autumn to 8.4 in spring, and Wali-Tangi Dam from 7.2 in winter to 7.6 in summer. A significant change in the temperature of waters is recorded in different seasons, especially summer (18.5 and 18.0 °C) and winter (1.0 and 1.5 °C) in Hanna Lake and Wali-Tangi Dam, respectively. Water temperature gradually decreased from the summer to winter season in both the waters, and there was almost no difference in temperatures of both. Higher turbidity was recorded in waters during spring and summer compared to autumn and winter. Generally, turbidity in Hanna Lake was slightly higher than Wali-Tangi Dam. The highest value of DO was 7.8 in both the waters in spring. Changes in DO and turbidity were not significant.

Winter is the rainy season in Quetta (December–March), contrary to the rest of Pakistan where the summer season receives the maximum rainfall due to the southwest monsoon. Although, the winter rainy season is relatively mild, however, helps to produce turbidity in both Hanna Lake and Wali-Tangi Dam in the winter season. Relatively higher values of turbidity observed in the summer may be attributed to recreational activities in water and melting of ice on mountains and subsequent flows. Turbidity has no direct health effects but can indicate the presence of disease-causing microorganisms (EPA 2002a). If the suspended particles have adhering organisms, there will be a potential risk of infection. On the other hand, turbulence contributes to the oxygenation of the water. DO is crucial for the survival of organisms and values less than 2 mg/l could result in the killing of fish (San et al. 2008). However, resistant elements of parasites do not require oxygen, because of being metabolically inactive. The highest number of parasites was found in the autumn season at both locations, i.e. 45 in Hanna Lake and 40 in Wali-Tangi Dam, when DO was the lowest and turbidity was low. An association between these factors and the proliferation of parasites may not be a true explanation. It may be associated with the post-summer dry times with a reduced volume of water in both locations. Reduced volume might have enumerated more numbers because of the concentration factor. Reduced volume of both of these waters seems to have resulted in low DO, owing to increased pollutants due to the concentration factor.

All the bacteriological parameters in both the waters decreased considerably in winter (Table 2, Figure 5). Overall, bacterial count recorded in Hanna Lake was higher than the Wali-Tangi Dam water. TC in Hanna Lake and Wali-Tangi Dam decreased up to 98.93 and 98%, respectively, in winter compared to summer. FC count decreased by 98.16 and 97.33% in

![Figure 4](http://iwaponline.com/jwh/article-pdf/doi/10.2166/wh.2022.291/1012863/jwh2022291.pdf)
winter, compared to summer in Hanna Lake and Wali-Tangi Dam, respectively. Similarly, *E. coli* was reduced by 98.55% in Hanna Lake and 98.89% in Wali-Tangi Dam compared to summer. Enterococci were not detected at all during winter in Wali-Tangi Dam and were reduced by 99.15% in Hanna Lake. This decrease can be attributed to the high sensitivity of bacteria towards temperature, which approaches near-zero in both the waters during winter in this area. During the winters of Quetta, tourists avoid visiting these recreational points, reducing human-induced contamination. Similarly, animal-induced contamination is also reduced by warm-blooded animals. These observations are contrary to observations recorded in the case of parasites, despite the concentration factor and reduced pollution input. Similarly, the bacterial concentration was high in summer despite the dilution factor due to an increased flow of water from melting ice. It shows the responsiveness of bacteria towards elevating temperature.

However, this decrease was not observed either in quantity or in variety in the case of parasitic elements, in both the waters in any season. In all seasons, at least four parasitic genera were detected at both locations. According to European Union Guidelines for recreational surface waters, based on bacterial indicators, the waters in Hanna Lake and Wali-Tangi Dam were fit for human recreational activities in the winter season. However, based on the presence of parasitic contamination, these waters were not fit for human exposure. In the presence of *Entamoeba* spp., Amoebas, *Microsporidium* sp., which were present in all the seasons including severe winter at both locations, these waters cannot be declared fit for human exposure. Hence, merely faecal indicator count is not the only useful tool to indicate the presence or absence of parasitic species in the aquatic environment and declare its fitness for human exposure.

### Possible candidate species for monitoring

In the present study, the most frequently occurring species of *Entamoeba* and *Microsporidium*, and summer metazoan *A. lumbricoides* could be possible candidates for detecting parasitic contamination in these surface freshwaters. *Entamoeba* cyst is about 10 times larger in size than the *Microsporidium* spores, hence, can be identified rather easily. *Entamoeba* cyst also does not need any staining in the identification process; therefore, a relatively semi-trained individual can also identify this parasitic element. For the spring and summer seasons, monitoring *A. lumbricoides*, which was persistently found specifically in these seasons in both environments, could be used as a candidate species for parasitic contamination. The size of this

### Table 2 | Counts of indicator bacteria in Hanna Lake and Wali-Tangi Dam in number/100 ml (average count of 3 months of each season)

| # | Parameter               | Hanna Lake                      | Wali-Tangi Dam                    |
|---|-------------------------|---------------------------------|-----------------------------------|
|   |                         | Spring  | Summer | Autumn | Winter | Spring  | Summer | Autumn | Winter |
| 1 | Total coliform (TC)/litre| $3.8 \times 10^3$               | $4.9 \times 10^3$               | $2.8 \times 10^3$               | $1.1 \times 10^2$  | $3.7 \times 10^3$               | $4.5 \times 10^3$               | $2.3 \times 10^3$               | $1.2 \times 10^2$             |
| 2 | Faecal coliform (FC)    | $8.5 \times 10^2$               | $9.8 \times 10^2$               | $5.8 \times 10^2$               | $1.8 \times 10^1$  | $7.1 \times 10^2$               | $7.5 \times 10^2$               | $3.5 \times 10^2$               | $2.0 \times 10^1$             |
| 3 | *E. coli*               | $5.5 \times 10^2$               | $6.9 \times 10^2$               | $4.2 \times 10^2$               | $1.0 \times 10^1$  | $3.5 \times 10^2$               | $4.5 \times 10^2$               | $2.1 \times 10^2$               | $5.0 \times 10^0$             |
| 4 | Enterococci (ENT)       | $5.1 \times 10^2$               | $5.9 \times 10^2$               | $3.9 \times 10^2$               | $5 \times 10^0$    | $2.9 \times 10^2$               | $3.8 \times 10^2$               | $2.0 \times 10^2$               | ND                              |

### Figure 5 | Seasonal pattern of bacterial counts in waters of Hanna Lake and Wali-Tangi Dam.
worm and its egg is large and can be easily identified. This parasite is ideal to be used to detect parasitic contamination during the summer recreational season and the tourists and all inhabitants of the area swimming in these waters can be readily alerted of the water quality. Ascariasis and amoebiasis have a high prevalence in Pakistan (Khan & Khan 2018). In these circumstances, the current study suggests _A. lumbricoides_ and _Entamoeba_ as indicator species to monitor these waters under resource scarce settings.

**CONCLUSIONS**

Total 11 parasitic elements belonging to 10 species were recorded in the present study including _Trichomonas_ sp. (trophozoite), _Isospora_ sp. (ocyst), _B. coli_ (cyst), _Cryptosporidium_ sp. (ocyst), _Entamoeba_ spp. (trophozoite), _Entamoeba_ spp. (cyst), Amoebas (trophozoite), _Microsporidium_ sp. (spore), _E. nana_ (cyst), _A. lumbricoides_ (egg), and _Giardia_ spp. (cyst) in Hanna Lake and Wali-Tangi Dam. Parasitic contamination remained persistent at both locations throughout the year independent of physico-chemical parameters and bacterial contamination. Reliance on bacterial presence only for monitoring of recreational waters could be a risk for tourists. _Entamoeba_ spp. and _A. lumbricoides_ are relatively easy to identify and could be used for surface water monitoring in these waters.

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**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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