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Identification of Aquatic Macro-invertebrates and Determination of *E. coli* and Heavy Metals in Rawal Lake, Pakistan

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ABSTRACT: *Water is an important constituent of life's support system. However, industrial growth, urbanization and anthropogenic activities have affected the quality of water bodies mostly in the world. The aim of the study was to assess the water quality of Rawal Lake, Pakistan, which is source of drinking water for the residents of Islamabad and Rawalpindi, using macro-invertebrates as pollution indicators, and through microbial and heavy metal analysis. For this purpose, macro-invertebrates and water samples were collected from Rawal Lake (Korang Nala Entrance), Rawal Lake (Centre) and from Rawal Lake (Spillway) points. The collected samples were preserved and transported to the Kinnaird College laboratory, where macro-invertebrates like caddisfly, water boatman, water bugs, gilled snails, mayflies etc. were identified with the help of identification guides and keys, while the presence of E. coli and heavy metals: Cadmium, Copper, Chromium, Cobalt, Nickel, Manganese and Zinc in the water samples was assessed. The results showed that pollution sensitive macro-invertebrates were mostly found in Rawal Lake (Centre), indicating that the water undergoes a self-cleaning process, whereas, water samples of Rawal Lake (Korang Nala Entrance), Rawal Lake (Spillway) had mostly pollution tolerant species, which signified that the water of these areas was moderately polluted. Simpsons' Index of Diversity for Rawal Lake indicated a high level of diversity in the area (0.85). Microbial analysis indicated the presence of E. coli in all the three sampling points of the lake. The levels of Cadmium (Korang Nala 0.007 ppm, Spillway 0.014 ppm) were found to exceed the World Health Organization permissible limits (0.005 ppm), in the water samples, however, the concentration values of other metals were within the permissible limits. Thus, it was concluded that the water of Rawal Lake was considered fit for drinking and other purposes in residential and commercial areas after being processed through a treatment plant.*

Keyword: Macro-invertebrates, Rawal Lake, Pollution, Heavy Metals, Drinking Water, *E. coli*

INTRODUCTION

Water and water resources are critical for ensuring a sufficient food supply and a healthy environment for all living things. Global freshwater demand has been quickly increasing as human populations and economies have grown. Water shortages significantly degrade biodiversity in both aquatic and terrestrial ecosystems, in addition to endangering human food supplies. Global population growth, climate change impacts, and lifestyle changes are putting increasing strain on our crucial water supplies, resulting in widespread water stress in many countries (Hatami, 2013).

Macro invertebrates are living organisms, found abundantly on the earth and are large enough to be seen with the naked eye. They include a variety of organisms like; leeches, mosquitoes, water bugs, midges, crustaceans, shrimps, snails, worms, crayfish and larvae of insects etc (Appenroth, 2010). There are two types of macro invertebrates i.e., terrestrial and aquatic. Aquatic macro invertebrates can be found in almost all wet environments including; rivers, lakes, marshes, wetlands, streams and pool habitats. They feed on plant litter, periphyton (organisms attached to submerged surfaces), aquatic detritus, and capture live prey (Chadde, 2005).

Macro invertebrates have an important role in the environment. Some species are important food sources for humans like crayfish and river shrimps. They are a primary part of the diet of fishes in fresh water environments and are often used as bait for recreational angling and fly-fishing. Macro invertebrates can play a valuable role as pollution indicators. They form an important constituent of an aquatic ecosystem and has functional importance in assessing the trophic status, changes in water quality and their available habitat (Rafia and Ashok, 2014).

Heavy metals are inherent elements of the earth's crust, having a specific density of more than 5 g/cm^3 (Järup, 2003; Singh et al., 2011). Examples include: Copper, Lead, Cadmium, Chromium, Manganese, Arsenic, Selenium, Nickel, Silver, Zinc, Aluminum, Cesium, Cobalt, Molybdenum, Strontium, Mercury and Uranium (McIntyre 2003). When present in extremely low quantities, these metals are required for maintaining certain biochemical and physiological activities in living organisms; nevertheless, when concentrations reach certain thresholds, they become toxic (Jaishankar et al., 2014; Nagajyoti et al., 2010).

Heavy metals enter the environment through natural processes as well as human actions. Soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control chemicals applied to crops and many more factors are all sources of heavy metals (Morais et al., 2012).

Ingestion and inhalation are the most common ways that humans are exposed to these metals. Working at or living near an industrial site that uses these metals and their compounds, as well as living near a site where these metals have been improperly disposed of, increases one's risk of exposure. Confusion, numbness, nausea, vomiting, headache, muscular pain, joint pain, weakness, and constipation are all symptoms associated with heavy metal poisoning, which can be acute or chronic (Baldwin and Marshall, 1999). Rawal Lake which is a source of drinking water for the residents of Islamabad and Rawalpindi (Ghumman, 2011). Therefore, the study was aimed to provide the information on the current state of the water quality using macro invertebrates as pollution indicators and through microbial and heavy metal analysis in the study areas so that regular monitoring and mitigation measures to improve the water quality can be implemented.

MATERIAL and METHODS

The samples were collected from three different locations of Rawal Lake (Fig. 1) i.e., Korang Nala Entrance, Centre of the Lake and near the Spillway Area in September 2019.

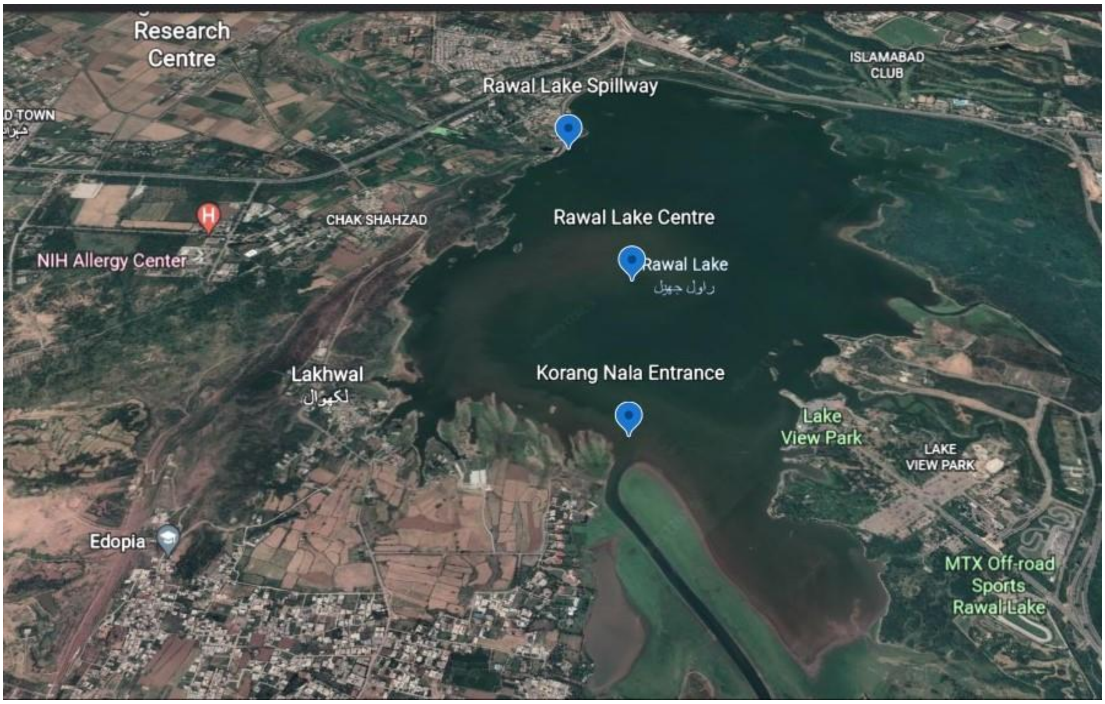

Fig. 1. Map of Sampling Sites of Rawal Lake

Sample Collection

The water samples were collected from the abovementioned selected sites in the plastic jars. In order to collect aquatic macroinvertebrates, dip nets with 0.6 mm mesh size were dragged in the water at the Entrance of Korang Nala, Centre of the Lake and then towards the Spillway. All contents of mesh were emptied into properly labelled plastic jars containing water from the same freshwater sites. The collected macro invertebrates were then separated, counted and preserved in a solution of 30% of Glycerin, 20% of Distilled Water, and 50% of 70% Alcohol and transported to Kinnaird College Laboratory for further analysis (Emmanuel et al. 2012).

Sample Identification

The samples were sorted out separately, photographed and identified with the help of identification guides and keys and their numbers and diversity was also noted. "A guide to the study of fresh-water biology" by Needham and Needham (Needham and Needham 19338), "A Community Water Quality Monitoring Manual for Victoria" by Kruger and Lubczenko (Victoria, 2012), "Invertebrate Identification Guide" by Robertson (Robertson et al., 2012) along with Zoological Experts at Kinnaird College for Women, Lahore and various online sources, provided great help in identifications.

Microbial Analysis

The microbial quality of water was assessed using *Escherichia coli* (*E. coli*), a simple, reliable, low-cost water quality test that can be used in resource limited settings, as indicators of fecal contamination of drinking water (Stauber et al. 2014). Mac Conkey Agar was freshly prepared and autoclaved for 30 minutes along with the apparatus. The agar plates were covered with 1 ml water samples from the three aforementioned sampling points using the spread plate method and were incubated for 24 hours at 37ºC. After incubation, presence and quantity of *E. coli* was determined in the water samples of the selected areas using the Colony Forming Unit (CFU) method (Smruti and Sanjeeda, 2012). Furthermore, biochemical tests: Catalase, Simmon's Citrate Agar, Indole Production, Nitrate reduction, Urease production, Methyl red tests and Presumptive tests were also performed to confirm the presence of *E. coli* (Soomro et al., 2002).

Heavy Metal Analysis

The water samples were prepared for digestion for Heavy Metal Analysis, with the help of Nitric acid (Uddin, Khalid et al. 2016). After digestion, the sample was diluted. Stock solutions of the standard salts were prepared, and the

Samples of water and macro invertebrates (Table 1) were collected from Rawal Lake and the following results were obtained

samples were later analyzed for the presence of heavy metals (Chromium, Cadmium, Cobalt, Copper, Nickel, Manganese and Zinc) using 210 A BUCK Scientific Atomic Absorption Spectrophotometer at different wavelengths i.e. Chromium (560 nm) Cadmium (396 nm), Copper (580 nm), Nickel (722 nm), Manganese (530 nm), Zinc (353 nm) and Cobalt (525-535 nm).

Data Analysis

Graph pad prism software for analysis. Column Statistics was applied using one-way ANOVA (non-parametric) and Bonferroni Test to compare all columns with 95% significance level.

RESULTS

Samples of water and macro invertebrates were collected and results were recorded (Table 1).

Table 1: Diversity of Macro invertebrates Found in Rawal Lake

Name	Rawal Lake (Korang Nala Entrance)	Rawal Lake (Centre)	Rawal Lake (Spillway)
Caddisfly	+	-	-
Creeping Water Bug	-	-	+
Dobsonfly	-	+	-
Freshwater Mussel	-	+	+
Gilled Snail	+	-	-
Mayfly larvae	+	+	-
Midge Larvae	-	+	+
Mosquito larvae	+	-	+
Pouch Snail	+	+	+
Predaceous Diving Beetle	-	+	+
Riffle beetle	-	+	-
Stonefly	+	-	-
Water Boatman	+	-	-
Water Scavenger Beetle Larvae	-	-	+
Whirligig Beetle	+	-	-

Table 2 showed the abundance of species found at Rawal Lake (Korang Nala Entrance, Centre and Spillway Area) sampling points and their pollution status and ecological linkages are presented in Table 3. The species found included: Caddisfly, Stonefly, Water Boatman, Whirligig Beetle, Gilled Snail, Dobsonfly, Freshwater Mussel, Mayfly larvae, Midge larvae, Pouch Snail, Predaceous Diving Beetle, Riffle Beetles, Creeping Water Bug, Mosquito Larvae and Water Scavenger Beetle Larvae (Faiz, 2020; Shelly et al., 2011; Ashfaq and Ashfaq, 2012; Niaz et al., 2019; Gondal et al., 2020; Kazmi and Ghory, 2019; Ganie et al., 2014; Parikh et al., 2021).

Table 2: Classification and Abundance Macro invertebrates Found in Rawal Lake

Genus	**Scientific Name**	**Common Name**	**Rawal Lake (Korang Nala)**	**Rawal Lake (Centre)**	**Rawal Lake (Spillway)**
Tricoptera	*Tricoptera*	Caddisfly	1	0	0
Ambrysus	*Ambrysus sp.*	Creeping Water Bug	0	0	13
Protohermes	*Protohermes motuoensis*	Dobsonfly	0	5	0
Lamellidens	*Lamellidens marginalis*	Freshwater Mussel	0	1	1
Melanoides	*Melanoides tuberculata*	Gilled Snail	1	0	0
Ephemeroptera	*Ephemeroptera*	Mayfly larvae	2	1	0
Dasineura	*Dasineura amaramanjarae*	Midge Larvae	0	1	1
Culex	*Culex quinquefasciatus*	Mosquito larvae	1	0	33
Indoplanorbis	*Indoplanorbis exustus*	Pouch Snail	7	1	1
Laccophilus	*Laccophilus inefficiens*	Predaceous diving beetle	0	36	1
Stenelmis	*Stenelmis pandiani*	Riffle beetle	0	20	0
Amphinemura	*Amphinemura schmidi*	Stonefly	1	0	0
Corixa	*Corixa substriata*	Water Boatman	20	0	0
Enochrus	*Enochrus esuriens*	Water Scavenger Beetle Larvae	0	0	5

Gyrinidae	*Gyrinidae*	Whirligig Beetle	2	0	0

Table 3: Pollution Status and Ecological Linkages of Macro invertebrates in Rawal Lake

Name	**Status**	**Ecological Linkages**
Caddisfly	Pollution intolerant	Prey of many kind of fish (Okano et al., 2017).
Creeping Water Bug	Pollution sensitive	Adults and nymphs are predacious on a variety of macro invertebrates (Chattha et al., 2018).
Dobsonfly	Pollution intolerant	Active predators feed on variety of stream invertebrates. Prey on aquatic immature mayflies, caddisflies, and stoneflies (Ishikawa et al., 2017).
Freshwater Mussel	Pollution sensitive	Filter feeder. Feed on Small,organic material in water taken in through siphon (Shafiullah et al., 2021).
Gilled Snail	Pollution intolerant	Scavenge dead animals, eat algae, fungi, animal feces, lichen Prey of birds, mammals, toads, turtles (Afshan et al., 2013).
Mayfly larvae	Pollution sensitive	Mayfly larvae feed on detritus and other plant materials. Prey on fish and parasitic round worm (Shelly et al., 2011).
Midge Larvae	Pollution tolerant	Prey for insects and fish (Memon et al. 2020).
Mosquito larvae	Pollution tolerant	Feed on organic debris and microorganisms. Prey of insects and fishes (Blaustein and Chase, 2007).
Pouch Snail	Pollution tolerant	The pouch snails are like vacuum cleaners of the stream because they feed on algae, aquatic plants and animal matter. Food for small vertebrates and invertebrates (Afshan et al., 2013).
Predaceous Diving Beetle	Pollution sensitive	In addition to cannibalism, these larvae also consume insects, snails, tadpoles, and fish. Food for birds and mammals (Hajek, 2006).
Riffle beetle	Pollution sensitive	Prey of insects, birds and fish (Elliott, 2008).
Stonefly	Pollution intolerant	Prey of fish and predator of invertebrates (Jalil and Mohamed, 1970).
Water Boatman	Pollution tolerant	Prey of frogs, fish, dragonflies, spiders and birds (Chattha et al., 2018).

Water Scavenger Beetle Larvae	Pollution sensitive	Capture a variety of aquatic invertebrates such as mosquito larvae and snails (Ojija et al. 2016).
Whirligig Beetle	Pollution tolerant	Prey for birds and fish (Shelly et al., 2011).

Simpson's Index of Diversity

Through Simpson's index formula i.e. $D_s = 1 - [\sum n_i (n-1)/N (N-1)]$ community diversity of Rawal Lake was measured (McIntyre, 1995). The range for Simpson index is 0-1 where scores close to 1 indicate high diversity and scores close to 0 indicate low diversity. Simpson's Diversity Index for Rawal Lake came out to be 0.85 (Table 2), while the species diversity of the individual sampling points calculated were: Rawal Lake (Korang Nala Entrance) 0.785, Rawal Lake (Centre) 0.60 and Rawal Lake (Spillway) 0.585.

Microbial Analysis

Growth of *E. coli* Colonies in Rawal Lake (Korang Nala Entrance) Water Sample (A), Rawal Lake (Centre) Water Sample (B), Rawal Lake (Spillway) Water Sample (C).

It was noticed that 488×10^2 colonies/ml of sample of *E. coli* were observed on the petri plates containing water sample from Korang Nala Entrance and 100×10^2 colonies/ml of sample were visible on the petri plate containing water sample collected from the centre of the lake. The samples collected from the spillway showed growth of 395×10^2 colonies/ml of sample of *E. coli.* Biochemical tests: Catalase, Simmon's Citrate Agar, Indole Production, Nitrate reduction, Urease production, Methyl red and Presumptive tests indicated the presence of *E. coli* in all three sampling areas of the lake (Table 4).

Table 4: Characterization of biochemical test

Biochemical Test	**Reaction (Korang Nala Entrance)**	**Reaction (Rawal Lake Centre)**	**Reaction (Spillway Area)**

Catalase	+ ve	+ ve	+ ve
Simmon's Citrate Agar	- ve	- ve	- ve
Indole Production	+ ve	+ ve	+ ve
Nitrate reduction	+ ve	+ ve	+ ve
Urease production	- ve	- ve	- ve
Methyl red	+ ve	+ ve	+ ve
Presumptive Test	+ ve	+ ve	+ ve

Heavy Metal Analysis

International Standards of Heavy Metal Concentration in Drinking Water by WHO/FAO and Concentration of Heavy Metals in the Selected Samples were compared (Table 5).

Table 5: Heavy Metal Concentration in Drinking Water by WHO/FAO and selected study sites

Sampling Sites/WHO/FAO Standard (ppm)	**Cr**	**Co**	**Ni**	**Zn**	**Cu**	**Mn**	**Cd**
WHO Standard	0.1	1.3^9	0.02	5	1	0.05	0.005
Rawal Lake (Korang Nala Entrance)	0.002	0	0.011	0.01	0.005	0.006	0.007
Rawal Lake (Centre)	0.002	0	0.015	0.08	0.003	0.005	0.004
Rawal Lake (Spillway)	0.001	0.002	0.016	0.01	0.004	0.005	0.014

The results (Table 5) depicted that the concentration of Chromium in water samples was less than 0.1 ppm, Cobalt in water samples was less than 1.3^9 , Nickel was less than 0.02 ppm, Zinc in the collected samples were below 5 ppm, respectively, according to the WHO/FAO standard concentrations of heavy metals in drinking water (WHO, 2004).

DISCUSSION

Both pollution tolerant and intolerant species were found near the Korang nala entrance sampling point, indicating that the site is contaminated due to mixing of sewage water carried along Korang Nala from households and factories. Pollution sensitive species were mostly found in the centre of the lake, implying that the water in the area is less polluted and is able to clean itself, by removing bacteria and debris from its surface, while the occurrence of pollution tolerant species near the spillway area signify that the water present there is polluted, as the area is connected to the boating site which is allowed for recreation and fishing (Irfan et al., 2020).

Simpson's Diversity Index for Rawal Lake (0.85), depicted a high species diversity. However, the species diversity of the individual sampling points showed high species diversity at Korang Nala Entrance (0.785) and above average diversity at Lake Centre (0.60) and Spillway (0.585).

Microbial analysis depicted the occurrence of *E. coli* in all three sampling regions of Rawal Lake was due to the mixing of Korang Nala water in the lake because it carries untreated sewage water from households and localities situated near its catchment area. Presence of higher number of colonies of *E. coli* in the samples collected from Korang Nala Entrance (488×10^2 colonies/ml of sample) was because the site is highly contaminated due to mixing of sewage water carried along Korang Nala from nearby homes and factories. Water sample collected from the centre of Rawal Lake, had lesser number of colonies (100×10^2 colonies/ml of sample) because the water has the capacity to clean itself, by removing bacteria and debris from its surface. Sample collected from spillway again showed a high rise in the number of *E. coli* colonies (395×10^2 colonies/ml of sample) because the site is heavily used for recreation and fishing, therefore the water of the area is polluted (Irfan et al., 2020).

The results of heavy metal analysis showed, the concentration of Cadmium in water samples exceeded the 0.005 ppm WHO/FAO standard concentration, while the remaining heavy metal concentrations were within the permissible limits. Recorded concentration of heavy metals was less than 1 ppm in all water samples except cadmium because these heavy metals are present in trace amounts in water bodies either naturally or due to acid rain. Increased concentration of cadmium in water was due to acidified soil nearby (Shi et al., 2016). However, level of cadmium in water can be controlled by using natural phosphates, liming or by using agricultural limestone. Heavy metals are accumulated in water due to addition of industrial waste, household waste (detergents, soaps), fumes and acid rain. These heavy metals if present in high concentrations enter in the food web through macro invertebrates which are preyed upon by secondary and tertiary consumers. These metals can also accumulate in fish and lead to dis-functioning of their organ systems. Eventually health issues in humans are reported as humans feed on fish and other animals in the same food web (Baharom and Ishak, 2015).

CONCLUSION

The water of Rawal Lake contains pollution tolerant macroinvertebrate species at the Korang Nala Entrance and Spillway sampling points, *E. coli* at the three sampling sites and high levels of cadmium, thus indicating that the water of the Lake is moderately polluted. However, the water passes through a treatment plant and undergoes a chlorination process, which makes it suitable for drinking before being supplied to the residents of Islamabad and Rawalpindi. For future prospect, regular monitoring and installation of improved treatment plant and septic tanks is advisable.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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