TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

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The Wetlands Diversity

Editors

Doru Bănăduc, Katrin Teubner & Angela Curtean-Bănăduc

Sibiu – Romania
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International Association for Danube Research	"*Lucian Blaga*" University of Sibiu	Ecotur Sibiu N.G.O.	Applied Ecology Research Center of *LB*US	Broward College, Fort Lauderdale	University of Vienna

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IN MEMORIAM

Klaus Werner Battes

(1943 – 2021)

Klaus Werner Battes was a Romanian ichthyologist of German nationality.

He was born on 12 October 1943, into a family of hard working Saxon farmers, in Sânpetru commune, Braşov County. The turmoil of the historical events of the Second World War and the conquering and domination of Eastern Europe by the communist Soviet Union of that time, led to the family losing their property including their house confiscated by the communists. They were relocated by force.

After education at primary and secondary schools in Mateiaş and Sânpetru communes, and high school in the city of Braşov, Battes finally graduated in 1967 from the Faculty of Biology, Geology and Geography, Biology-Zoology Section, at Babeş-Bolyai University, Cluj-Napoca. He obtained his PhD in biology at Alexandru Ioan Cuza University of Iaşi (Romania) in 1978 with thesis entitled "Growth rate and respiratory metabolism in fish raised under natural conditions, in retention ponds and in directed intensive growth".

Blown by the winds of history to another Romanian province, Moldavia, Battes, a strong character and hard working person, obtained the unanimous respect of his colleagues and friends, and settled his new family, including two daughters one of whom, Dr. Karina Battes, followed the same natural sciences call; she is now enjoying a successful carrier in hydrobiology at the university where her father took his PhD.

Within his rich career, he increased his prestige through outstanding scientific results obtained in ichthyology at the Biological and Geographical Research Station "Stejarul" of Alexandru Ioan Cuza University of Iaşi, the Laboratory of Aquaculture and Aquatic Ecology of Piatra Neamţ, and the Biology Department, Faculty of Sciences, at Bacău University.

In the last teaching position he held, he succeed in reaching both the minds and the souls of his students, guiding them firmly but delicately in nature sciences education and careers.

His important international scientific collaborations were based on close and long-lasting relations with researchers and teachers from countries including Austria, Germany, Hungary, and Portugal.

His main research fields included fish physiology and biology in natural and anthropic water bodies; salmonids and cyprinids aquaculture in super-intensive farming systems; fish nutrition; salmonids reproduction; ichthyofauna monitoring; aquatic ecosystems ecology, and integrated management.

After a lifetime of hard work, Battes left behind for the science of ichthyology nine registered inventions, over 60 research reports, over 10 books and chapters in books, and over 150 scientific papers. He brought his professional experience to editorial boards of natural science journals such as: Scientific Studies and Research, Biology, Bacău, Acta Ichtiologica Romanica, and Acta Oecologica Carpatica.

He was also an active member of the Romanian Association of Scientists; Romanian Academy of Scientists; Gheorghe Ionescu-Sişeşti Academy of Agriculture and Forestry Sciences; Romanian Limnological Society; Romanian Ichthyological Society; Romanian Aquaculture Society; Romanian Ecology Society, and others.

Prof. ***Klaus Werner Battes's*** exceptional talent for nature-related sciences, high moral standards, dedication, hard and organized working, and excellent human relations with his family, friends, colleagues and students due to his kind natural sensibility and genuine modesty, characterised him through his whole life, and make him a rare example for all the people who had the good fortune to meet him. An excellent and rare example to be followed ...

The Editors

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand allready the presence of the medium and long-term significant change in the "average weather" all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

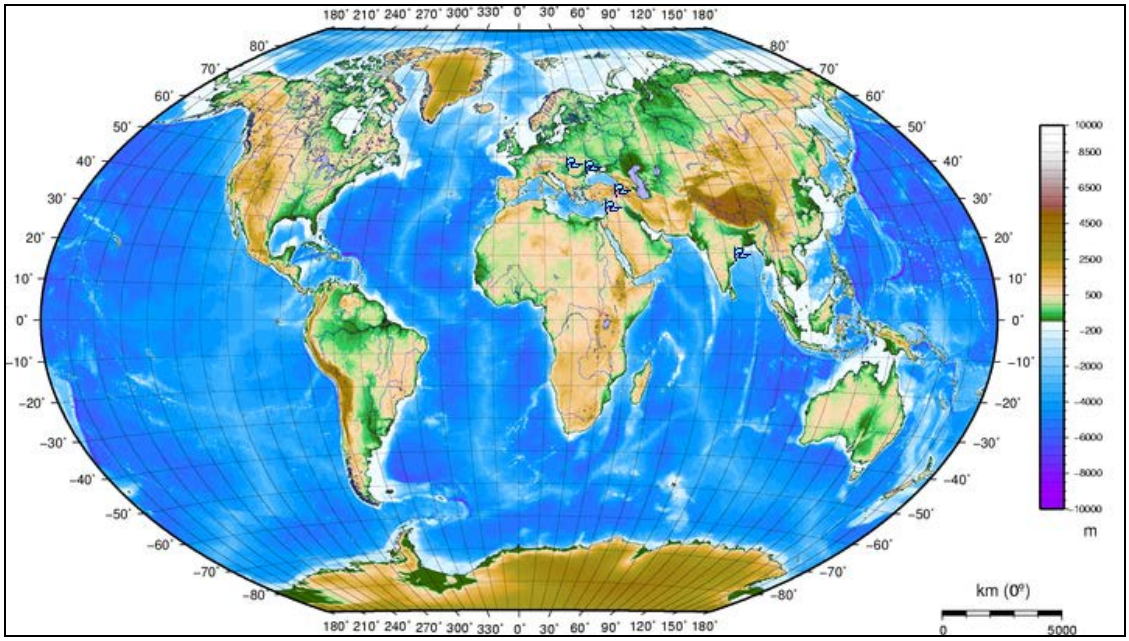
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch three annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2017.

The therm wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources. **Marine/Coastal Wetlands** – Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal. **Inland Wetlands** – Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands –** Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue the annual sub-series (*Wetlands Diversity*) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly "images" of a perishing world …

This volume included variated original researches from diverse wetlands around the world.



The subject areas (▱) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The editors would like to express their sincere gratitude to the authors and the scientific reviewers whose work made the appearance of this volume possible.

The Editors

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ECOLOGICAL CONDITIONS OF THE WATERCHESTNUT (*TRAPA NATANS* L.) IN THE DANUBE DELTA (ROMANIA)

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KEYWORDS: environment quality indicator, ecological gradients, type of water bodies, site diversity, waterchestnut communities.

ABSTRACT

The diversity of water body types in the Danube Delta offers appropriate ecological niches for the colonisation of frequently large stands of the waterchestnut (*Trapa natans*). Their phytocoenoses were observed in slowly running and standing waters from clear, sediment-poor, to turbid and sediment-rich waters on muddy ground.

Trapa natans occurs in standing, and slowly running, waters and is well adapted to fluctuation of water level changes. The water dynamics is responsible for the composition of accompanying species of the phytocoenoses. The particular zonation, demonstrated by a cross section shows the adaption to the structure and the water flow of certain water bodies. Comparing older and newer research data, a decline of the populations of waterchestnuts became visible.

ZUSAMMENFASSUNG: Ökologische Bedingungen der Gewöhnlichen Wassernuss (*Trapa natans* L.) im Donaudelta (Rumänien).

In der Vielfalt der Gewässertypen des Donaudeltas findet auch die Wassernuss (*Trapa natans* L.) ihre entsprechenden ökologischen Nischen in denen sie sich in größeren Beständen entwickeln kann. Ihre Phytcoenosen wurden sowohl und in langsam fließenden, aber auch in stehenden Gewässern vorgefunden, die von klar und arm an Sedimenten, bis hin zu sehr sedimentreichen, turbiden Gewässern auf stärker schlammigen Grund reichen. Die Wasserstandsdynamik bedingt das Vorkommen bestimmter Begleitarten in der Zusammensetzung der Phythocoenosen. Ein Vergleich älterer und neuerer Aufnahmedaten, zeigt trotz der beachtlichen, vorhandenen Populationen einen deutlichen Rückgang der Wassernussvorkommen.

REZUMAT: Condițiile ecologice ale ciulinilor de baltă/cornaci (*Trapa natans* L.*)* în Delta Dunării (România).

În diversitatea mare a tipurilor de corpuri de apă, ciulinii de baltă, numiți și cornaci (*Trapa natans* L.) își găsesc nișe ecologice corespunzătoare pentru a se dezvolta deseori în grupări foarte mari. Fitocenozele lor au fost găsite atât în ape lin curgătoare, dar și în ape stătătoare, de la relativ limpezi și sărace în sedimente, până la ape turbide bogate în sedimente și cu fundul de lac cu soluri nămoloase. Dinamica apelor Dunării condiționează existența anumitor specii însoțitoare în componența fitocenozelor. Comparând cercetări din trecut cu cele recente, se constată totuși un declin vizibil al suprafețelor populate în trecut de *Trapa natans*.

INTRODUCTION

Waterchestnut (*Trapa natans* L.) an Eurasian continental-Mediterranean species (Ciocârlan, 1994; Oberdorfer, 2001, 2009; Sârbu et al., 2013) occurs as a bottom-rooted species in slowly flowing and standing eutrophic waters. According to Cook (1996) – seen in a global view – it occurs in seasonally summer-warm to tropical waters of the Old World i.e. Eurasia, being naturalized in North America. The distribution map of the waterchestnut shows two distinctly separated areas (Walter and Straka, 1970), one from Central to Eastern Europe reaching to the upper part of the delta of the Volga River (Losev et al., 1998) and the other including South-East Asia and the southern Far East (Walter and Straka, 1970). But in the European part of its large distribution area, the waterchestnut populations are increasingly in decline - in particular in Central and Western Europe - and in danger of extinction. This is a result mainly of change of ecological conditions including water quality and loss of natural habitats. That is the reason why the species was included as a first measure for its conservation in Europe in the "Convention on the Conservation of European Wildlife and Natural Habitats", known as the Bern Convention. It was opened for signature at the 33th Ministrial Conference on September 1979 in Bern, Switzerland, and came into force on 1 June 1982. The Appendix I with the list of strictly protected flora species includes among others the waterchestnut (*Trapa natans*). In Germany this species is not only included in the Red List of species, but also in the Red List of endangered plant communities (Rennwald, 2000). These data correspond to the occurrences of *Trapa natans* in Central Europe, but in the Red Book of vascular plants of Romania this species is not included (Dihoru and Negrean, 2009).

The actual decline of the waterchestnut (*Trapa natans*) in Central Europe has been proved by comparing its distribution in the same area (old branches of the Upper Rhine near to Rastatt) as a monitoring place over a longer time period, including the years 1978, 1989/90, 2005 and 2015 (Werling et al., 2017). From the once larger area covered by waterchestnuts the populations decreased to a few individuals and finally disappeared from the area. A local re-introduction was not successful. The decrease was stated in more areas of the Upper Rhine, but apart from the above mentioned causes such as loss of habitats and water quality, no others are mentioned. There are some assumptions stating that increasing populations of muskrats using the nuts as nutrition contributed to their disappearance.

At present, the only larger occurrence exists on the Upper Rhine area in the old Rhine meander "Rußheimer Altrhein" a Nature Reserve north of the town of Karlsruhe (Adler, 2020). The waterchestnut occurred in the Eastern part of Germany (Rothmaler, 1976), for example on the Elbe river and the Elbe Havel and other smaller river systems as can be followed on the distribution map no. 1852 (Benkert et al., 1996). But, in the most mentioned places, the populations of the waterchestnut fell or disappeared completely.

Going back to historical data, including as well pollen analyses, the waterchestnut has a long history in Europe, extending during the warm periods after the last Ice Age (Walter and Straka, 1970; Schloss, in verbis 2021). From a historical-cultural point of view, the species had an important role as it was the first plant used in Europe for production of flour in the Mesolithic /end of Ice age cca. 9600 BC to the Neolithic 5600 BC (Poschlod, 2017).

On the Upper Rhine, natural pollen deposits have been analysed in old meanders by Schloss S. (in manuscript). On the area of the Sewensee, a lake of Southern Vosges below the Ballon d'Alsace (altitude 500 m), the same author reported copious nuts of *Trapa natans* and pollen deposits. These are dated ca 6000 years BC VI and continuously until the postglacial warm time (Firbas, 1949, 1952), Schloss supposing that it was cultivated (letter from 02.04.2021, unpublished data). According to Plinius Gaius Secuwadus in A.D. 23 the Thracian peoples also used *Trapa natans* centuries later for flour production (Drăgulescu, 2008).

Looking at the occurrences of the waterchestnut in South-Eastern Europe, especially in the Danube Delta, the situation differs from that of Western and Central Europe, as the ecological conditions are more appropriate for a larger spread of this species. But, also in this area changes took place, as they can be followed comparing older data from the Lower Danube and maps of the Danube Delta before and after hydrotechnical works (Gâștescu and Știucă, 2008). E. Țopa (1957) mentioned *Trapa natans* L. from the Danube Delta in the Dunavăț area before any hydrotechnical works took place, the Sulina area, at Sf. Gheorghe in the "Turkish canal", near to the „Oleanca" island and in the Letea area. According to Neagu-Godeanu (1973) the waterchestnut association Trapetum natantis Müller and Görs 1960, composed by a species with a large ecological amplitude, having its main distribution area in the upper part of the Delta, i.e. the area of Rusca, Carasuhat, Șontea-Sireasa, Gorgova-Uzlina, Eracle and the Old Danube. Occurrences on the lower part of the delta are not mentioned. A subspecies *muzzanensis* (Jaggi) Schinz of *Trapa natans* is also mentioned in the Danube Delta (Doroftei and Covaliov, 2013). Data, concerning ecological gradients in the Danube Delta with occurrences of aquatic macrophytes in different vegetation types include also the vegetation type *Trapa natans* based on TWINSPAN classification (Coops and Hanganu, 2000).

MATERIAL AND METHODS

Over more than twenty years in the Danube Delta, studies of the water macrophytes and their ecological requirement have been realized. A special focus was given for *Trapa natans* L., which occurs in this area in different type of water bodies with various ecological niches. Samples of 5 x 5 m were taken along chosen stretches according to the method of Braun-Blanquet with the seven degree abundance-dominance scale (Braun-Blanquet, 1964; Borza and Boșcaiu, 1965). Along some channels cross sections where realized. In the Eracle and Lopatna channels with near natural structure of the borders, then cross sections from one to the other border and on a distance from ten to ten meters between the cross sections were realized showing the succession of different plant communities. As well cross sections from ten to ten meters were realized on the Sf. Gheorghe branch with its lateral channel Gârla de Mijloc (middle channel), for studying the succession of vegetation from the open water mirror with waterchestnut (*Trapa natans*) in smaller and larger strips or cover of reeds and forests.

The study area at all include parts of the Șontea-Fortuna complex (Fortuna, Crânjală, Lacul Rotund, the Gorgova-Isac-Uzlina complex with Taranova, and Perivolovca), parts of the Matița-Merhei complex (Lopatna, Eracle, Babina, and Lungu lakes), the Litcov Channel area (locally), the Caraorman Channel and Sf. Gheorghe with Gârla de Mijloc and Meleaua Sacalin.

The samples were grouped in tables with indicator values for nitrogen and temperature (Ellenberg et al., 2001), as being important for the development of waterchestnut communities.

The samples taken from the different sites are included in phyto-coenological tables grouped according to the studied stretches of running water in natural channels, the so-called Gârlas, more artificial canals and lakes. From the indicator values according to Ellenberg et al. (2002), Sanda et al. (1983); Sârbu et al. (2013) are included in the table those of temperature (T) and nitrogen (N) as they are of significant importance for the existence and development of waterchestnut communities. The nomenclature of species is given according to Sârbu et al. (2013), Ciocârlan (2009), and Oberdorfer (2001).

The samples were used as well for detailed studies concerning the species composition and structure of the phytocoenoses and their ecological requirements. All the phytocoenoses were considered in strong relation to the hydro-morphological dynamics, the grain size of sediments, the water quality and the succession of the vegetation along ecological gradients.

RESULTS AND DISCUSSION

Over the large area of the Danube Delta, the waterchestnut *Trapa natans* L. phytocoenoses were observed in slowly running and standing waters from clear, sediment-poor, to turbid and sediment-rich waters on muddy ground. Due to the structure of its ramified stems supporting more leaf rosettes the waterchestnut is able – as has been observed – to adapt to changing water levels. It occurs in waters with various sediment loads but also locally in clear waters. The depth of the water is between 20 cm to around 130 cm, but the given data are varying between 20 and 200 cm (Krausch, 1965; Coops and Hanganu, 2000). The study areas presented below demonstrate the large ecological conditions in which *Trapa natans* occurs.

The Șontea – Fortuna complex

In this complex our attention were focused on the area from Crânjală Channel and Lake Fortuna to the polder Fortuna including Lake Rotund, called by the local people „Lake Cruglic" (Lipovan language around the lake). In a part of it the waterchestnut covers a large area. The increasing abundance-dominance by phytocoenoses, with 100% cover and only a few accompanying species is in strong relation with the inflow of sediment-rich Danube water through the open connection with the Sulina main branch of the Danube at Mila 26 (mile 26) flowing further through the channels of the polder Fortuna, so that the river water loaded with silt has access to Lake Rotund. In this sediment-rich water, the waterchestnut finds good conditions for a luxuriant development (Figs. 1 and 2; Tab. 1, column 1-2, 5-6, 9-12).

Figure 1: Rotund Lake 2008.

Figure 2: Rotund Lake 2011.

With its morphological construction, the roots of the water nuts play also a filtering function between clear and turbid water in the lake. Apart from the dominant waterchestnut on the border of this cover, there are also transition stages, where *Trapa natans* is represented only with lower abundance-dominance values, being more or less replaced by the dominant species *Ceratophyllum demersum* and large covers of *Salvinia natans* (Tab. 1). With the continuous inflow of sediment-rich water, the lake will be completely silted up. In the area of Crânjală Channel and Lake Fortuna, *Trapa natans* has been less frequent, but the inflow from the channel with sediment-rich water was as easily visible by the occurrence of strips of flowering rush (*Butomus umbellatus*), also an indicator species for siltation processes.

Table 1: Phytocoenoses of waterchestnut (*Trapa natans*) and accompanying species in the area of Fortuna Lake, polder Fortuna including Rotund Lake.

		Number of sample	1	2	3	4	5	6	7	8	9	10	11	12
		sampling 7.09.2006	*	*	*	*	*	*	*	*				
		sampling 23.06.2007									*	*		
		sampling 10.09.2011											*	*
T	N													
		Species												
7	8	*Trapa natans*	5	5	2	2	4	4	2	3	4	5	5	5
6	6	*Spirodela polyrrhiza*	.	.	.	.	.	+	+	+	+	+	.	+
5	5	*Potamogeton crispus*	1	+	1	.	.	.	+	.	.	.	.	.
7	8	*Ceratophyllum demersum*	+	+	.	3	3	2	4	4	+	.	.	+
6	6	*Hydrocharis morsus-ranae*	+	.	.	+	.	.	.	.	.	.	.	.
6	8	*Typha latifolia*	+	.	.	+	.	.	.	.	.	.	.	.
5	7	*Phragmites australis*	+	.	.	+	.	.	.	.	.	.	.	.
6	6	*Nuphar lutea*	.	3	1	.	.	.	.	.	.	.	.	.
6	7	*Elodea nuttalii*	.	.	.	.	+	.	.	.	.	.	.	.
5	6	*Lemna minor*		+	.	+	+	+	+	.	.	+	+	+
x	8	*Potamogeton pectinatus*	.	.	.	.	.	.	2	+	.	+	4	3
8	7	*Salvinia natans*	+	+	5	3	+	1	2	.	.	.	.	.
6	6	*Lemna trisulca*	+	.	.	.	+	+	.	.	.	.	.	.
6	5	*Nymphaea alba*	.	.	.	.	.	.	.	.	.	.	.	+
8	8	*Azolla filiculoides*	.	.	.	+	+	+	.	.	.	.	.	.
6	7	*Potamogeton lucens*	.	.	.	+	.	+	.	.	.	.	.	.
6	6	*Najas marina*	.	.	.	.	.	.	.	.	.	+	.	.
8	8	*Lemna gibba*	.	.	.	.	.	.	.	.	.	.	+	+

Fortuna samplings: 7.09.2006, 1-4, Crânjală Channel, lake Fortuna and polder Fortuna; 7.09.2006: 5-8: lake Rotund western side; 9 and 10: Fortuna: 23.06.2007, samples 11 and 12: Rotund lake eastern side, 10.09.2011.

The Gorgova, Gorgoștel, and Uzlina complex

The above mentioned lake area presents, in comparison with the area of Fortuna, more diversified phytocoenoses (Schneider-Binder, 2018). In that area, we can find in the Gorgoștel Lake, together with the waterchestnut, the yellow water lily (*Nuphar lutea*), mentioned as characteristic for the subassociation of the ass. Trapetum natantis Müller et Görs 1960 (Philippi, 1969), described as representative from the Upper Rhine in Germany. The same association is large represented in the Danube Delta (Krausch, 1965; Popescu et al., 1997).

Table 2: Phytocoenoses of waterchestnut (*Trapa natans*) and accompanying species in the area of Gorgoștel, Gorgova, and Uzlina; 1 g = mouth of the channel from Gorgoștel Lake to Perivolovca; 2 g = lake Gorgoștel, 12.06.2003 depth 0.80 m, on the border *Phragmites australis*; 3 g = lake Gogorgoștel, moderate water current, 12.06. 2003; 4 g = lake Gorgoștel, depth 1.80 m – 0.30 m, 25.09.2004; 5 g = lake Gorgoștel, 25.09.2004; 6 Gt = lake Gorgovăț, 26.09.2004; 7 G lake Gorgova, 15.06.2003; 8 Gc = Gorgovăț Channel, 15.06. 2003; 9 Gt = lake Gorgovăț, 15.06. 2003; 10 Gt = lake Gorgovăț; 11 T = Taranova Channel, 25.09.2004; 12 and 13 U = lake Uzlina, 16.06.2003; 14 U-P = lake Uzlina near to the Uzlina Channel (moderate flowing water), 16.06.2003. Abbreviations: g = lake Gorgoștel; Gt = lake Gorgovăț; G = lake Gorgova; Gc = Gorgovăț Channel; T = Taranova Channel near to the Taranova Lake; U = Uzlina Lake; U-P = transition from Uzlina Lake to the Uzlina Channel.

		Number of sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14
		Sampling point	g	g	g	g	g	Gt	G	Gc	Gt	Gt	T	U	U	U-P
T	N															
		Species														
7	8	*Trapa natans*	5	3	4	4	3	5	5	3	3	5	5	+	5	1
6	6	*Spirodela polyrrhiza*	+	.	.	.	.	+	.	+	+	+	+	+	+	.
5	5	*Potamogeton crispus*	1	+	1	.	.	.	.	+	.	.	.	.	.	.
7	8	*Ceratophyllum demersum*	2	+	+	+	+	.	.	2	+	+	.	+	.	.
6	6	*Hydrocharis morsus-ranae*	+	.	.	+	.	.	.	.	.	.	.	.	.	+
6	8	*Typha latifolia*	+	.	.	+	.	.	.	.	.	.	.	.	.	.
5	7	*Phragmites australis*	+	.	.	+	.	.	.	.	.	.	.	.	.	.
6	6	*Nuphar lutea*	.	3	1	.	.	.	.	.	.	.	.	.	.	.
6	7	*Elodea nuttalii*	.	.	.	.	.	+	.	.	.	.	.	.	.	.
5	6	*Lemna minor*	.	.	.	.	.	+	.	+	.	+	+	.	+	.
x	8	*Potamogeton pectinatus*	.	.	.	.	.	.	+	3	+	.	+	3	.	2
8	7	*Salvinia natans*	.	.	.	+	+	.	.	+	.	.	.	.	.	.
6	6	*Lemna trisulca*	.	.	.	.	.	.	.	+	.	.	.	.	.	.
6	7	*Myriophyllum spicatum*	.	.	.	.	+	.	.	.	.	.	.	.	.	.
6	5	*Nymphaea alba*	.	.	.	.	.	.	.	.	.	.	.	+	.	+

The presence in the samples 5-7, 9-10, and 12 of the fennel or sago pondweed (*Potamogeton pectinatus*) adapted to running waters of different speed indicate the striking distance of the Uzlina Channel, flowing inside the Uzlina Lake and influencing the species composition of the waterchestnut phytocoenoses. The presence of 15 different aquatic macrophytes is a proof for a more diversiefied variant of *Trapa natans* phytocoenoses, in general represented by a low number of accompanying species.

The Lopatna, Eracle channels, Lungu Lake area, and Babina Lake (of Matița and Merhei area)

The Lopatna and Eracle (partly) channels are part of the old natural channel system in this area of the delta. Without or with small human intervention they are representative for the succession of the vegetation from one to the other border of the channels. Along the bordering reeds we can follow the ribbon-like disposed phytocoenoses in a relatively clear water with only a small sediment load. Seen in cross section, the reed bed of both sides is followed by a belt with dominant yellow water lily (*Nuphar lutea*), with floating watermoss (*Salvinia natans*) between the leaves, and in the middle part with slowly running water the waterchestnut is dominant (Figs. 3 and 4). The Babina Lake north of the channel system is also representative (Fig. 5).

Table 3: Phytocoenoses of *Trapa natans* in the Lopatna-Eracle area; Lo = Lopatna channel; L-E intersection; E= Eracle channel; Lu = lake Lungu. Sample 1, 2, 3 Lopatna channel, 4 = Lopatna Eracle channels intersection, 5 and 6 = Eracle channel, 6 = lake Lungu, all samples from 9.09.2011.

		Number of sample	1	2	3	4	5	6	7
		Sampling point	Lo	Lo	Lo	L-E	E	E	Lu
T	N								
		Species							
7	8	*Trapa natans*	5	5	4	3	5	5	5
8	7	*Salvinia natans*	.	1	1	.	+	+	+
7	8	*Ceratophyllum demersum*	.	.	.	.	.	.	.
6	6	*Stratiotes aloides*	+	+	1	.	.	.	.
6	6	*Hydrocharis morsus-ranae*	.	.	+	.	.	.	.
6	6	*Nuphar lutea*	.	.	.	3	+	+	.
6	5	*Potamogeton nodosus*	.	.	.	.	.	+	.
5	7	*Phragmites australis*	.	.	.	.	+	+	.

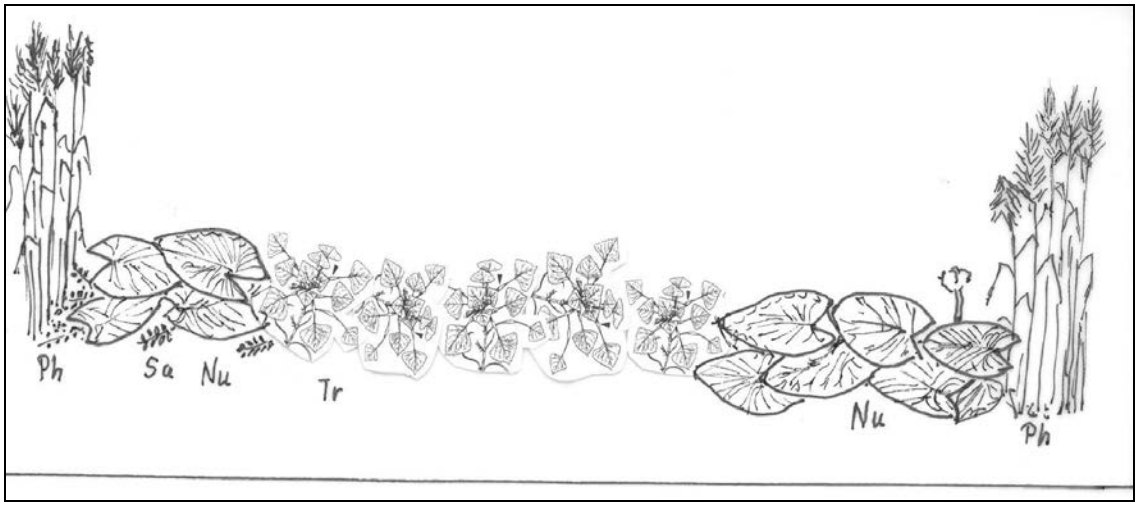

Figure 3: Cross-section through the macrophyte vegetation of Lopatna channel (Ph = *Phragmites australis*, Sa = *Salvinia natans*, N = *Nuphar lutea*, Tr = *Trapa natans*).

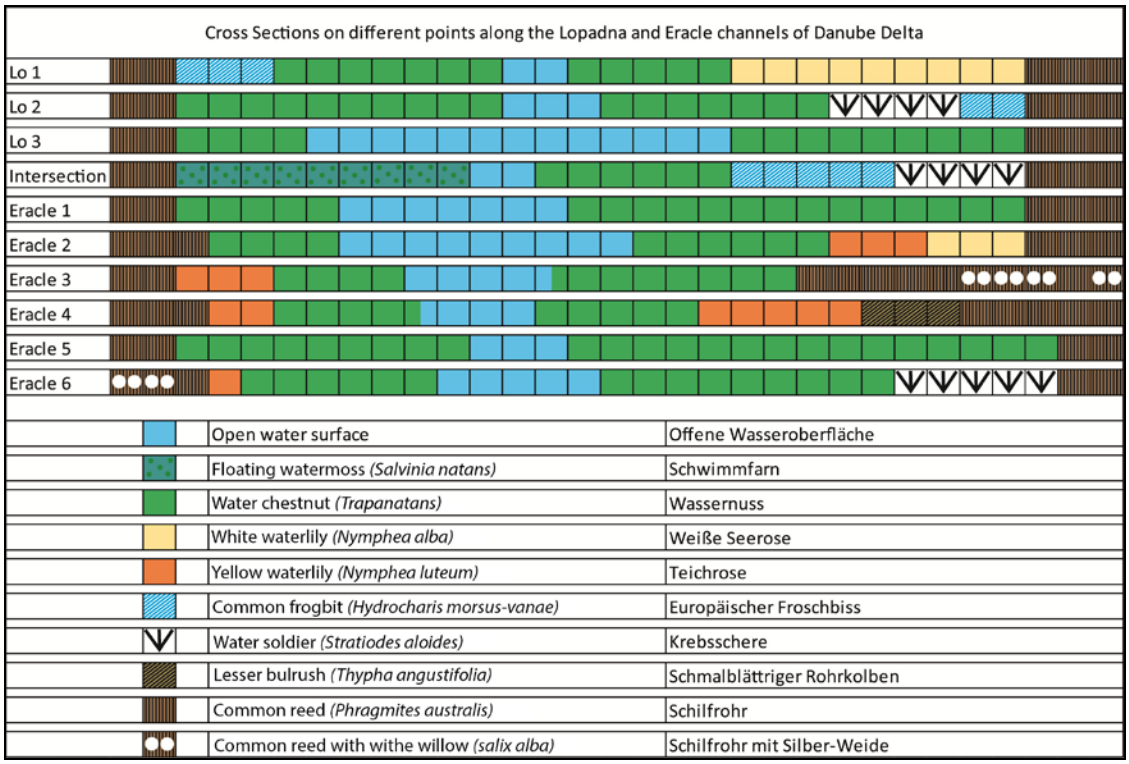

Figure 4: Cross sections (10) from one to the other border, showing the succession of different plant communities.

Figure 5: Babina Lake with common reed stands bordered by large carpets of *Trapa natans* (covering degree 80-100) north of the Lopatna-Eracle channel system, July 2021; photo and sampling Becker I. (KIT).

Old Danube (M), channel Crișan, channel Litcov (eastern part), lake Iacob (Iacub), channel Caraorman.

The *Trapa*-type vegetation is well represented in this old stream and canal system, including the Old Danube, the Crișan channel, Litcov and Caraorman channels (Fig. 6). In that area is mentioned as well a large occurence of Trapa-type vegetation in the Obretinu lake (Coops and Hanganu, 2000). The number of accompanying species is low (Tab. 4) and beside the dominant *Trapa natans*, only *Salvinia natans* is more frequent.

Table 4: Phytocoenoses of *Trapa natans* in the area of the old Danube (Dunărea Veche), channel Crișan, channel Litcov, lake Iacub and channel Caraorman; DV = Dunărea Veche/Old Danube, on the „M" the cutted former Meanders of the Sulina branch; Iac (= lake Iacob); Cri = channel Crișan; Lit = Litcov Channel eastern part, sample 4, 25.09.2004; Car = channel Caraorman; Date of sampling: 1-4 = 9.09.2011); 5-7 = (12.09.2018).

		Number of sample	1	2	3	4	5	6	7
		Sampling point	DV	Lo	Lo	L-E	E	E	Lu
T	N								
		Species							
7	8	*Trapa natans*	2	5	5	5	5	4	3
5	7	*Butomus umbellatus*	2	.	.	.	.	.	.
8	7	*Salvinia natans*	1	5	1	.	+	2	.
6	6	*Sagittaria sagittifolia*	.	.	.	+	1	.	.
6	5	*Spirodela polyrrhiza*	.	.	+	+	.	.	2
5	7	*Phragmites australis*	.	.	.	1	.	.	.

Figure 6: Caraorman Channel with carpets of waterchestnuts (*Trapa natans*) in summer aspect, with interloking carpets of fringed water lily (*Nymphoides peltata*), 12.08.2021 (Photo Isabell Becker KIT, institute for wetlands ecology).

Danube branch Sf. Gheorghe, Gârla de mijloc, Gârla Turcească, Meleaua Sacalin

A particular occurrence of *Trapa natans* can be observed on the Sf. Gheorghe branch with its ramifications Gârla de Mijloc, Gârla Turcească and finally the Meleaua Sacalin with its part named Sacalinu Mic. In that area the water flow broadens out on a large surface with low water level fluctuation, so that a special succession (seen as cross-section) of vegetation is developed downstream the fluvial-km 5. It is represented by *Trapa natans* on the open water side followed by common reed (*Phragmites australis*), locally with lesser bulrush (*Typha angustifolia*) and black alder (*Alnus glutinosa*) on a small river bank, being an indicator for the small fluctuation of the water level in that area. Downstream of km 5 the small fringe of *Trapa natans* and reeds broaden out more and more (Fig. 7) being visibly a large siltation process. In that area the waterchestnut grows in some abundance with overlapping of the rosettes in very low waters and finally it covers the muddy soil. Characteristic for these siltation areas, near to the waterchestnut is also the locally dominant flowering rush (*Butomus umbellatus*) (Fig. 7).

Figure 7: Small fringe of *T. natans* and ribbon-like small stands of common reed (*Phragmites communis*) and black alder (*Alnus glutinosa*) on fluvial-km 5 of the Sf. Gheorghe branch.

Table 5: *Trapa natans* Phytocoenoses on Sf. Gheorghe branch, Gârla de Mijloc.

		Number of sample	1	2	3	4	5	6	7	8	9	10
T	N											
		Species										
7	8	*Trapa natans*	5	5	5	5	5	4	3	2	1	2
6	7	*Butomus umbellatus*	.	.	+	+	.	2	3	3	3	2
6	7	*Typha angustifolia*	1	.	+	1	.	.	.	.	.	.
5	7	*Phragmites australis*	+	.	.	.	1	.	.	.	2	.
6	7	*Salix alba*	.	.	.	.	.	.	.	.	1	.

All samples 1-10 are from the Sf. Gheorghe branch downstream km 5, Gârla de Mijloc from ten to ten m distance; Date: 06.09.2011.

Figure 8: Carpets of *Trapa natans*, lesser *Typha angustifolia* and *Salix alba* stands.

Figure 9: Ribbon- like stands of *Trapa natans* (first front), *Butomus umbellatus*, lesser *Typha angustifolia*, *Phragmites australis* and *Salix alba* on the left back.

Figure 10: *Trapa natans* covering as large carpets with high abundance-dominance the remained water surfaces in the siltation area (06.09.2011).

CONCLUSIONS

The waterchestnut, a thermophilous species, is widely spread in the Danube Delta, populating different sites mostly as a monodominant species. The number of accompanying species is low, and accounts for 3-7 species, all with small cover degree. Temperature and nitrogen are very important for the waterchestnut, which requires in its flowering time and the growing of fruits temperatures which exceed +20°C. The water is mostly turbid, but the species occurs as well in moderate turbid and also in clear waters. On the bottom of the most sites where the species occurs the mud is rich in humus, but poor in calcium carbonate.

Trapa natans occurs in standing and slowly running waters and is well adapted to fluctuation of water level changes. The water dynamics is responsible for the composition of accompanying species as it was stated be the inflow of the Uzlina Channel into the Uzlina Lake and as well by the phytocoenoses in the Lopatna Channel. The particular zonation, demonstrated by a cross section of the vegetation in the Lopatna area, shows the adaption to the structure and the water flow of a certain water body. Primarily the populations of waterchestnut (*Trapa natans*) are bound to the inflow area of a water stream into a lake, as has been observed by the inflow in Lakes Rotund, Obretinu Mic and Uzlina. In this case the waterchestnuts have a large cover degree and develop a high biomass.

From a historical view it is possible to state some changes in the whole water macrophyte vegetation, including also the waterchestnuts populations. This fact became mostly visible when considering older data (Țopa, 1957; Neagu-Godeanu, 1973, 1975; Munteanu and Curelariu, 1996; Gâștescu and Știucă, 2008), and also newer data according to our recent researches. By comparing these data, the long term trends are coming out indicating a decline of the populations. This is caused by different hydrotechnical works such as dyking, drainage and transformation of wetlands into agricultural lands and fishpolders as it shows for example the data of the former Dunavăț and Holbina area, but also the area of Fortuna, as well of Babinia and Cernovca (Schneider et al., 2008).

Apart from the above mentioned facts, a reduction of *Trapa natans* populations on their typical sites is caused by very low water levels, high temperatures, evaporation and the drying out of lakes, consequences also of the climate changes.

Considering the occurrences of *Trapa natans* in South-eastern Europe, even if at some sites a decline is visible in comparison with those in Central and Western Europe, it emerges clearly that site conditions in South-eastern Europe such as continentality, higher temperatures in summer time and other site conditions in general, all together assure a larger ecological amplitude and more diverse site conditions than for populations of the species in Central and Western Europe.

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PLANTS, MOSSES, CHAROPHYTES, PROTOZOAN, AND BACTERIA WATER QUALITY INDICATORS FOR ASSESSMENT OF ORGANIC POLLUTION AND TROPHIC STATUS OF CONTINENTAL WATER BODIES

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KEYWORDS: plants, mosses, charophytes, protozoan, and bacteria, ecological preferences, water quality, organic pollution, bioindicators.

ABSTRACT

This paper presents data compilation for indicator species of organic pollution and trophic state of continental waters. Information was collected from research papers, monographs, electronic resources, and our research. Altogether 316 species of higher plants, plants, mosses, charophytes, protozoan, and bacteria from 11 taxonomical phyla are represented with ecological preferences for saprobity indicators with saprobity index (S) and indicators of trophic state. This comprehensive data can be used for the purpose of aquatic ecosystem assessment and monitoring of water quality based on bioindication methods.

ZUSAMMENFASSUNG: Pflanzen, Moose, Charophyten, Protozoen und Bakterien als Indikatoren für die Beurteilung organischer Verschmutzung und trophischen Zustand kontinentaler Gewässer.

Dieses Papier präsentiert eine Datenzusammenstellung für Indikatorarten der organischen Verschmutzung und des trophischen Zustands kontinentaler Gewässer. Informationen wurden aus Forschungsarbeiten, Monographien, elektronischen Ressourcen und unserer eigenen Forschung gesammelt. Insgesamt sind 316 Arten höherer Pflanzen, Moose, Charophyten, Protozoen und Bakterien aus 11 taxonomischen Phyla mit ökologischen Präferenzen für Saprobitätsindikatoren mit Saprobitätsindex (S) und Indikatoren für den trophischen Zustand vertreten. Diese umfassenden Daten können für die Bewertung aquatischer Ökosysteme und die Überwachung der Wasserqualität auf der Grundlage von Bioindikationsmethoden verwendet werden.

REZUMAT: Plante, mușchi, carofite, protozoare şi bacterii, indicatori ai calității apei pentru evaluarea poluării organice și a stării trofice a corpurilor de apă continentale.

Această lucrare prezintă o compilare a datelor pentru speciile indicatoare de poluare organică și a stării trofice a apelor continentale. Informațiile au fost colectate din lucrări de cercetare, monografii, resurse electronice și propriile noastre cercetări. În total, 316 specii de plante superioare, mușchi, carofite, protozoare și bacterii din 11 phylumuri taxonomice sunt reprezentate cu preferințele ecologice pentru indicatorii de saprobitate cu indicele de saprobitate (S) și indicatorii stării trofice. Aceste date pot fi utilizate în scopul evaluării ecosistemului acvatic și al monitorizării calității apei pe baza metodelor de bioindicaţie.

INTRODUCTION

The biological variables, proposed by the European Union Water Framework Directive (European Parliament, 2000) as quality elements for the classification of the ecological status of waters included composition and abundance not only of algae and cyanobacteria but also other aquatic flora (i.e., macroalgae and angiosperms) (Ponti et al., 2009). Macrophytes, the large group of aquatic plants including Charophytes macroalgae, bryophytes, and vascular plants, are essential groups for assessing the water quality of surface waters. Because they can become very abundant, often showing the dominance of those species that are usually related to trophic states of water bodies (Pešić et al., 2020). The bioindication method, based on analyzing the response of biota to changes in environmental conditions, is of great significance in estimating the effect of pollution on aquatic ecosystems (Schindler, 1987; Kent, 2000; Fennessy et al., 2001; Mack, 2001; USEPA 2003; Reiss and Brown, 2005). Saprobity index S can be calculated for the macrophytes community and then incorporated into the water quality assessment system, especially for organic pollution (Savitskaya, 2017; Zueva and Bobrov, 2018). Assessment of anthropogenic impact by macrophytes is used for the lakes and wetlands (Poikane et al., 2018). And it is also a developed system with combined assessment methods for the diatoms, invertebrates, and other aquatic inhabitants (Friedrich et al., 1996; Hering et al., 2006).

Our work in collecting the environmental preferences of aquatic species has a long history. First of all, we paid attention to the ecological characteristics of algae and cyanobacteria, collecting information from solid published works of reliable authors and combining information into a database. The work continued for more than 30 years, and data were compiled for 11 groups of indicators in various indicator systems. Currently, part of this array of data on the ecological preferences of algae and cyanobacteria includes 9,450 species (Barinova and Fakhima, 2017). It was published in two books, the indicator tables are in English and are available via electronic link in references (Barinova et al., 2006, 2019). In this work, we have set the task to collect indicative data on other organisms inhabiting the aquatic environment. The material turned out to be so large that we considered it logical to divide into two parts, each of which combined into different publications. Currently, both these parts are ready for publication. The ecological preferences of aquatic invertebrates will be presented in the next volume of Transylvanian Review of Systematical and Ecological Research (24.1, 2022). Here, we present descriptions and results that combine reference data on indicator species from different phyla of aquatic plants, mosses, and other organisms that are not algae, cyanobacteria, and invertebrates. It can serve as an indicator of water quality to assess the impact of pollution on aquatic ecosystems.

We aimed to compile the list of aquatic species inhabitants in continental water bodies from plants, mosses, some protozoan, and bacteria with each species preferences for different level of water organic pollution and trophic state.

MATERIAL AND METHODS

The data collected on ecological preferences of aquatic species of plants, mosses, protozoan, and bacteria were taken from the available nine monographs, published papers, and electronic resources (Nevo and Wasser, 2000 [1]; Schneider and Melzer, 2003 [9]; Marvan et al., 2005 [2]; Haury et al., 2006 [8]; Porter, 2008 [4]; Jäger, 2010 [7]; Nagengast and Kuczyńska-Kippen, 2015 [5]; Becker et al., 2016 [3]; and Trbojević et al., 2020 [6]). The number in square brackets corresponds to the reference number in the environmental preferences table. Each species information was collected and inserted into the table, the data is classified according to categories of bioindication (Barinova, 2017a). The ecological

characteristics of species are grouped according to the following significant variables: trophic state and water saprobity with self-purification zones according to Sládeček (1973), and species-specific Index saprobity S.

The ecological preferences of each taxon are usually described in different sources, from which we took all the available information and then summarized it for each indicator. In the data integration process, we provided the indicator values mentioned in the reference, and if there were several data, then the highest of them. For example, if there were different values of the species-specific index of saprobity, then we gave the highest. On the other hand, if different values of the trophic category are indicated for the same species, then the highest was tacked; for example, from the mesotrophic and eutrophic, the eutrophic was chosen.

Integrated data about saprobity is defined in a scale of water quality with the relationship between saprobity index S and water quality category (Romanenko et al., 1990; Barinova, 2017a) (Tab. 1).

Table 1: Relationship between Water Quality Class, Rank, Index of Saprobity S, and self-purification zones.

Water Quality Class	Self-purification zone	Rank	EU Color code	Index Saprobity S	Saprobity zone
1	1	1	Blue	0-0.5	xenosaprobity
2	2a	2	Green	0.5-1.0	β-oligo-saprobity
2	2b	3	Green	1.1-1.5	α-oligo-saprobity
3	3a	4	Yellow	1.6-2.0	β'-meso-saprobity
3	3b	5	Yellow	2.1-2.5	β''-meso-saprobity
4	4a	6	Orange	2.6-3.0	α'-alpha-meso-saprobity
4	4b	7	Orange	3.1-3.5	α''-alpha-meso-saprobity
5	5a	8	Red	3.6-4.0	β-polysaprobity
5	5b	9	Red	> 4.0	α-polysaprobity
6	6	9	Black	> 4.0	transsaprobity

Index S community tolerance to the organic matter enrichments can be calculated on the base of collected data about species-specific index S. With the following equation, where S is the index of saprobity for the community; s_i is the species-specific saprobity index; a_i is the species frequency values (Eq. 1):

$$S = \sum_{i=1}^{n}(s_i.a_i) \Big/ \sum_{i=1}^{n}(a_i)$$

Equation 1

Trophic state preferences data is compared to the bioindicators category of this parameter (Barinova, 2017a) in the system started by Herman Van Dam in 1994 (Van Dam et al., 1994).

Species-indicators names are adopted to the modern taxonomic system with the help of available online sources (Cavalier-Smith, 1998, 2006; GBIF; WoRMS; Guiry and Guiry, 2021).

RESULTS AND DISCUSSION

As a result of collecting and integrating data about ecological preferences of aquatic plants, mosses, protozoans, and bacteria analyzed data from nine sources of references for 316 species (Tabs. 2-8) were analyzed. In all tables the same abbreviations were used: saprobity groups: x – xenosaprob, x–o – xeno–oligosaprob, o–x – oligo–xenosaprob, x–b – xeno–betamesosaprob, o – oligosaprob, o–b – oligo–beta–msosaprob, b–o – beta–oligosaprob, o–a – oligo–alpha–mesosaprob, b – beta–mesosaprob, b–a – beta–alpha–mesosaprob, a–o – alpha-oligosaprob, a – alpha-mesosaprob, i – i-eusaprob. Trophic state groups: ot – oligotraphentic; om – oligo–mesotraphentic; m – mesotraphentic; me – meso–eutraphentic; e – eutraphentic; o–e – oligo–eutraphentic; he – hypereutraphentic. "–" property is unknown. Their reference number is in square brackets and is the same in the reference list.

Table 2: Index saprobity S, saprobic zone groups, and group of trophic states for species of aquatic Bigyra with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Bigyra				
1.	*Bicosoeca dinobryoidea* Lemmermann 1914	2.00	b	–	[2]
2.	*Bicosoeca kepneri* Reynolds 1927	1.60	b-o	–	[2]
3.	*Bicosoeca lacustris* James-Clark, 1867	2.00	b	–	[2]
4.	*Bicosoeca mitra* Fott, 1946	2.00	b	–	[2]
5.	*Bicosoeca oculata* Zacharias, 1894	2.00	b	–	[2]
6.	*Bicosoeca ovata* Lemmermann, 1914	2.00	b	–	[2]
7.	*Bicosoeca pascheri* Conrad	2.50	b-a	–	[2]
8.	*Bicosoeca petiolata* (Stein F.) Pringsheim E. G. 1946	2.50	b-a	–	[2]
9.	*Bicosoeca socialis* Kent W. S. 1871	2.00	b	–	[2]
10.	*Bicosoeca campanulata* Bourrelly 1953	2.70	a-o	–	[2]
11.	*Bicosoeca conica* Lemmermann, 1914	2.00	b	–	[2]
12.	*Bicosoeca crystalline* Skuja 1956	2.70	a-o	–	[2]
13.	*Bicosoeca cylindrical* (Lackey) Bourrelly, 1951	1.60	b-o	–	[2]
14.	*Bicosoeca irregularis* (Pascher 1942) Bourrelly 1951	2.50	b-a	–	[2]
15.	*Bicosoeca planctonica* Kisselev 1931	1.80	o-a	–	[2]
16.	*Bicosoeca ruttneri* Wawrik	2.00	b	–	[2]
17.	*Bicosoeca synoica* Skuja, 1956	2.00	b	–	[2]
18.	*Poteriodendron petiolatum* Stein F., 1878	2.50	b-a	–	[2]
19.	*Pseudobodo minimus* Ruinen, 1938	3.00	a	–	[2]

Table 3: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Katablepharidophyta and Chlorobi with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Katablepharidophyta				
1.	*Katablepharis hyalurus* Skuja	3.00	a	–	[2]
2.	*Katablepharis notonectoides* Skuja	3.00	a	–	[2]
3.	*Katablepharis ovalis* Skuja	3.00	a	–	[2]
4.	*Katablepharis phoenikoston* Skuja	3.00	a	–-	[2]
	Chlorobi				
1.	*Ancalochloris perfilievii* Gorlenko and Lebedeva 1971	5.80	m	–	[2]
2.	*Chlorobacter symbioticum* Lauterborn, 1915	4.50	i	–	[2]
3.	*Chlorobacter vantieghemii* Pringsheim	4.50	i	–	[2]
4.	*Chlorobium limicola* Nadson, 1906	6.00	m	–	[2]
5.	*Chlorobium limicola* f. *thiosulfatophilum* (Larsen, 1952) Pfennig and Truper, 1971	6.00	m	–	[2]
6.	*Chlorobium chlorochromatii* Vogl et al., 2006	6.00	m	–	[2]
7.	*Chlorobium phaeobacteroides* Pfennig, 1968	6.00	m	–	[2]
8.	*Chlorobium phaeovibrioides* Pfennig 1968	6.00	m	–	[2]
9.	*Chlorobium vibrioforme* Pelsh, 1936	6.00	m	–	[2]
10.	*Chlorochromatium glebulum* Skuja, 1956	4.50	i	–	[2]
11.	*Chloroflexus aurantiacus* var. *mesophilus* Pivovarova et Gorlenko, 1976	5.80	m	–	[2]
12.	*Chloroflexus aurantiacus* Pierson and Castenholz, 1974	0.00	x	–	[2]
13.	*Chloronema giganteum* Dubinina and Gorlenko, 1975	5.70	m	–	[2]
14.	*Chloronema spiroideum* Dubinina and Gorlenko, 1975	5.90	m	–	[2]
15.	*Chloronostoc abbreviatum* Pascher, 1925	5.30	m	–	[2]
16.	*Chloroplana vacuolata* Dubinina et Kuznetsov, 1976	5.50	m	–	[2]
17.	*Microchloris nadsonii* Pringsheim 1953	5.50	m	–	[2]

Table 4: Index saprobity S, saprobic zone groups, and group of trophic states for species of aquatic Chloroflexi with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Chloroflexi				
1.	*Bacterium chlorophyllophorum* Winberg et Sivko 1952	6.00	m	–	[2]
2.	*Clathrochloris hypolimnica* Skuja, 1956	4.50	i	–	[2]
3.	*Clathrochloris* Witt et al., 1989	5.90	m	–	[2]
4.	*Cylindrogloea bacterifera* Perfiliev, 1914	6.00	m	–	[2]
5.	*Cylindrogloea solitaria* Skuja 1964	5.50	m	–	[2]
6.	*Pelodictyon aggregatum* Perfil'ev, 1914	4.50	i	–	[2]
7.	*Pelodictyon clathratiforme* (Szafer, 1911) Lauterborn, 1913	5.90	m	–	[2]
8.	*Pelodictyon luteolum* (Schmidle, 1901) Pfennig et Truper, 1971	4.50	i	–	[2]
9.	*Pelodictyon parallelum* (Szafer, 1910) Perfiliev, 1914	4.50	i	–	[2]
10.	*Pelodictyon phaeum* Gorlenko, 1972	5.80	m	–	[2]
11.	*Pelogloea bacillifera* Lauterborn, 1915	5.50	m	–	[2]
12.	*Pelogloea chlorine* Lauterborn, 1913	4.50	i	–	[2]
13.	*Pelosphaera rotans* Lauterborn, 1915	5.50	m	–	[2]
14.	*Prosthecochloris phaeoasteroides* Puchkova et Gorlenko, 1976	5.80	m	–	[2]
15.	*Sorochloris aggregate* Pascher 1925	5.10	m	–	[2]

Table 5a: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Proteobacteria and Ascomycota species with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Proteobacteria				
1.	*Allochromatium warmingii* (Cohn, 1875) Imhoff et al., 1998	5.40	m	–	[2]
2.	*Amoebobacter bacillosus* Winogradsky, 1888	5.70	m	–	[2]
3.	*Amoebobacter granula* Winogradsky, 1888	5.90	m	–	[2]
4.	*Amoebobacter pendens* (Molisch 1906), Pfennig and Trüper, 1971	5.70	m	–	[2]
5.	*Amoebobacter roseus* Winogradsky, 1888	5.00	m	–	[2]
6.	*Blastochloris viridis* (Drews and Giesbrecht 1966)	4.20	i	–	[2]
7.	*Chromatiopsis cinerea* Skuja, 1948	4.80	i	–	[2]
8.	*Chromatiopsis maior* Skuja	4.30	i	–	[2]
9.	*Chromatium buderi* Trüper and Jannasch, 1968	5.40	m	–	[2]
10.	*Chromatium fallax* (Warm.) Kolkw.	5.50	m	–	[2]
11.	*Chromatium gliscens* (Ehrenb.) Kolkw.	5.70	m	–	[2]
12.	*Chromatium gracile* Strzeszewski, 1913	6.00	m	–	[2]
13.	*Chromatium linsbaueri* Gicklhorn, 1921	5.90	m	–	[2]
14.	*Chromatium minus* Winogradsky, 1888	6.00	m	–	[2]
15.	*Chromatium minutissimum* Winogradsky, 1888	5.60	m	–	[2]
16.	*Chromatium molischii* (Bersa, 1926) van Niel, 1948	5.60	m	–	[2]
17.	*Chromatium okenii* (Ehrenberg, 1838) Perty, 1852	5.40	m	–	[2]
18.	*Chromatium vanda* Osnitzkaya and Chudina, 1978	5.80	m	–	[2]
19.	*Chromatium vinosum* (Ehrenberg, 1838) Winogradsky, 1888	5.60	m	–	[2]
20.	*Chromatium violacens* Perty, 1852	5.60	m	–	[2]
21.	*Chromatium warmingii* (Cohn, 1875) Migula, 1900	5.40	m	–	[2]
22.	*Ectothiorhodospira shaposhnikovii* Cherni et al. 1969	5.70	m	–	[2]
23.	*Lamprocystis gelatinosa* (Winogradsky 1888) Migula 1900	5.90	m	–	[2]
24.	*Lamprocystis rosea* (Kütz.) Drouet F.E., Daily W.A.	5.60	m	–	[2]
25.	*Lamprocystis roseopersicina* (Kutzing, 1849) Schroeter, 1886	5.00	m	–	[2]
26.	*Lamprocystis rubra* (Miyoshi) Migula	5.90	m	–	[2]
27.	*Lamprocystis symbiotica* Ponomarev	5.90	m	–	[2]
28.	*Lamprocystis violacea* (Miyoshi) Migula	5.90	m	–	[2]

Table 5b: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Proteobacteria and Ascomycota species with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Proteobacteria				
29.	*Magnetospirillum fulvum* (van Niel 1944) Hördt et al. 2020	6.00	m	–	[2]
30.	*Magnetospirillum molischianum* (Giesberger 1947) Hördt et al. 2020	6.00	m	–	[2]
31.	*Pararhodospirillum photometricum* (Molisch 1907) Lakshmi et al. 2014	6.00	m	–	[2]
32.	*Pelochromatium roseoviride* (Gorlenko and Kuznetsov, 1971)	5.80	m	–	[2]
33.	*Pelochromatium roseum* Lauterborn, 1913	5.70	m	–	[2]
34.	*Rhodobacter capsulatus* (Molisch 1907) Imhoff et al. 1984	4.80	i	–	[2]
35.	*Rhodoblastus acidophilus* (Pfennig 1969) Imhoff 2001	4.70	i	–	[2]
36.	*Rhodocyclus purpureus* Pfennig 1978	6.00	m	–	[2]
37.	*Rhodomicrobium vannielii* Duchow and Douglas 1949	5.80	m	–	[2]
38.	*Rhodopedia tetras* Skuja	5.10	m	–	[2]
39.	*Rhodopseudomonas gelatinosa* (Molisch 1907) van Niel 1944	5.60	m	–	[2]
40.	*Rhodopseudomonas globiformis* Pfennig 1974	5.80	m	–	[2]
41.	*Rhodopseudomonas issatchenkoi* Osnick.	5.90	m	–	[2]
42.	*Rhodopseudomonas palustris* (Molisch 1907) van Niel 1944 emend. Venkata Ramana et al. 2012	5.60	m	–	[2]
43.	*Rhodopseudomonas sphaeroides* van Niel 1944	4.80	i	–	[2]
44.	*Rhodopseudomonas sulfoviridis* Keppen and Gorlenko 1975	6.00	m	–	[2]
45.	*Rhodopseudomonas sulphidophila* Hans. et Veldk.	5.80	m	–	[2]
46.	*Rhodopseudomonas vannielii* Scard.	5.70	m	–	[2]
47.	*Rhodospirillum rubrum* (Esmarch, 1887) Molisch, 1907	5.50	i	–	[2]
48.	*Rhodospirillum tenue* Pfennig 1969	5.80	m	–	[2]
49.	*Thiocapsa pfennigii* Eimhjellen 1970	5.90	m	–	[2]
50.	*Thiocapsa roseopersicina* Winogradsky 1888	5.90	m	–	[2]
51.	*Thiocystis gelatinosa* (Winogradsky 1888) Pfennig and Truper 1971	5.40	m	–	[2]
52.	*Thiocystis rufa* Winogradsky 1888	5.90	m	–	[2]
53.	*Thiocystis violacea* Winogradsky 1888	5.90	m	–	[2]

Table 5c: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Proteobacteria and Ascomycota species with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Proteobacteria				
54.	*Thiodictyon bacillosum* (Winogradsky 1888) Pfennig and Truper 1971	5.70	m	–	[2]
55.	*Thiodictyon elegans* Winogradsky 1888	5.90	m	–	[2]
56.	*Thiopedia rosea* Winogradsky, 1888	4.80	i	–	[2]
57.	*Thiopolycoccus ruber* Winogradsky 1888	5.90	m	–	[2]
58.	*Thiosarcina rosea* (Schroeter) Winogradsky	5.90	m	–	[2]
59.	*Thiospirillum jenense* (Ehrenberg 1838) Migula 1900	4.80	i	–	[2]
60.	*Thiospirillum rosenbergii* (Warming) Migula 1972	5.90	m	–	[2]
61.	*Thiospirillum rufum* (Perty) Migula 1900	5.70	m	–	[2]
62.	*Thiospirillum sanguineum* (Ehrenberg) Winogradsky 1888	5.90	m	–	[2]
	Ascomycota				
1.	*Hyalobotrys hypolimnicus* Skuja 1964	4.50	i	–	[2]

Table 6a: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Bryophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Bryophyta				
1.	*Amblystegium fluviatile* (Hedw.) Schimp	2.50	b-a	–	[2]
2.	*Amblystegium riparium* (Hedw.) Schimp	1.80	o-a	–	[2]
3.	*Amblystegium tenax* (Hedw.) C.E.O. Jensen	0.50	x-o	–	[2]
4.	*Calliergon cuspidatum* (Hedw.) Kindb.	1.20	o	–	[2]
5.	*Calliergonella cuspidata* (Hedw.) Loeske	1.20	o	–	[2]
6.	*Chiloscyphus polyanthus* (L.) Corda	0.50	x-o	–	[2]
7.	*Chiloscyphus rivularis* (Schrad.) Loeske	1.40	o-b	–	[2]
8.	*Cinclidotus aquaticus* (Hedwig) Bruch and Schimper	1.10	o	–	[2]
9.	*Cinclidotus fontinaloides* (Hedw.) P. Beauv.	1.10	o	–	[2]
10.	*Cinclidotus nigricans* (Brid.) Wijk and Margad.	1.80	o-a	–	[2]
11.	*Cinclidotus riparius* (Host ex Bridel) Arnott	1.80	o-a	–	[2]
12.	*Cirriphyllum crassinervium* (Tayl.) Loeske and Fleisch.	1.20	o	–	[2]
13.	*Cratoneuron commutatum* (Brid.) G. Roth	0.10	x	–	[2]
14.	*Drepanocladus aduncus* (Hedw.) Warnst.	1.40	o-b	–	[2]
15.	*Eurhynchium crassinervium* (Taylor in Mackay) Schimper	1.20	o	–	[2]
16.	*Fissidens arnoldii* Ruthe	1.90	o-a	–	[2]
17.	*Fissidens crassipes* Wilson ex Bruch and Schimp.	1.80	o-a	–	[2]
18.	*Fontinalis antipyretica* Hedw.	1.30	o	–	[2]
19.	*Grimmia alpicola* (Thériot) H. Crum	1.70	b-o	–	[2]
20.	*Hygroamblystegium fluviatile* (Hedw.) Loeske	2.50	b-a	–	[2]
21.	*Hygroamblystegium irriguum* (Hook. and Wilson) Loeske	0.50	x-o	–	[2]
22.	*Hygroamblystegium tenax* (Hedw.) Jenn.	0.50	x-o	–	[2]
23.	*Hygrohypnum alpinum* (Lindb.) Loeske	0.20	x	–	[2]
24.	*Hygrohypnum luridum* (Schleicher ex Bridel) C. E. O. Jensen	0.50	x-o	–	[2]
25.	*Hygrohypnum ochraceum* (Turn. ex Wils.) Loeske	0.50	x-o	–	[2]
26.	*Hygrohypnum palustre* Loeske	0.50	x-o	–	[2]
27.	*Jungermannia atrovirens* Dumort.	0.90	x-b	–	[2]
28.	*Jungermannia cordifolia* Hook.	1.50	o-b	–	[2]

Table 6b: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Bryophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state gr.	References
29.	*Jungermannia exsertifolia cordifolia* (Dum.) Vana	1.50	o-b	–	[2]
30.	*Jungermannia lanceolata* (K. Muell.) Buch	0.90	x-b	–	[2]
31.	*Leptodictyum riparium* (Hedw.) Warnst.	1.80	o-a	–	[2]
32.	*Leskea polycarpa* Ehrh. ex Hedwig	2.60	a-o	–	[2]
33.	*Marchantia polymorpa* L.	1.00	o	–	[2]
34.	*Marsupella aquatica* (Lindb.) Schiffn.	0.50	x-o	–	[2]
35.	*Marsupella emarginata* (Ehrh.) Dum.	0.70	o-x	–	[2]
36.	*Marsupella emarginata aquatica* (Lindenb.)	0.50	x-o	–	[2]
37.	*Marsupella sphacelata* (Lindenb.) Dumort.	0.50	x-o	–	[2]
38.	*Palustriella commutata* (Hedw.) Ochyra	0.10	x	–	[2]
39.	*Pellia endiviifolia* (Dicks.) Dumort.	1.00	o	–	[2]
40.	*Pellia fabbroniana* Raddi	1.00	o	–	[2]
41.	*Phaeoceros carolinianus* (Michx.) Prosk.	0.80	x-b	–	[2]
42.	*Phaeoceros laevis* (L.) Prosck.	0.80	x-b	–	[2]
43.	*Philonotis fontana* (Hedw.) Brid.	0.30	x	–	[2]
44.	*Platyhypnidium riparioides* (Hedw.) Dixon	1.00	o	–	[2]
45.	*Platyhypnidium rusciforme* (Neck.) Fleischn.	1.00	o	–	[2]
46.	*Rhynchostegium riparioides* (Hedw.) Cardot	1.00	o	–	[2]
47.	*Riccia fluitans* L.	1.30	o	–	[2]
48.	*Riccia glauca* L.	1.30	o	–	[2]
49.	*Ricciocarpus natans* (L.) Corda	1.40	o-b	–	[2]
50.	*Scapania undulata* (L.) Dumort.	0.80	x-b	–	[2]
51.	*Schistidium agassizii* Sull. et Lesq.	1.70	b-o	–	[2]
52.	*Schistidium alpicola* (Hedw.) Limpr.	1.70	b-o	–	[2]
53.	*Solenostoma crenulatum* (Sm.) Mitt.	0.60	o-x	–	[2]
54.	*Sphagnum* sp.	1.70	b-o	–	[2]
55.	*Syntrichia latifolia* (Hartm.) Hueben.	1.70	b-o	–	[2]
56.	*Thamnobryum alopecurum* (Hedw.) Nieuwland ex Gangulee	0.20	x	–	[2]
57.	*Thuidium tamariscifolium* (Hedw.) Lindb.	0.80	x-b	–	[2]
58.	*Thuidium tamariscinum* (Hedw.) Schimp.	0.80	x-b	–	[2]
59.	*Trichocolea tomentella* (Ehrb.) Dumort.	0.70	o-x	–	[2]

Table 7a: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Magnoliophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Magnoliophyta				
1.	*Acorus calamus* L.	1.50	o-b	he	[2], [5]
2.	*Anacharis canadensis* (Michx.) Planch.	2.10	b	–	[2]
3.	*Baldingera arundinacea* (L.) Dumort.	2.10	b	–	[2]
4.	*Batrachium aquatile* (L.) Dumort	2.20	b	–	[2]
5.	*Batrachium carinatum* Schur	2.60	a-o	–	[2]
6.	*Batrachium circinati* (Sibth.) Fr.	–	–	me	[5]
7.	*Batrachium fluitans* (Lam.) Wimm.	1.70	b-o	–	[2]
8.	*Berula angustifolia* (L.) Koch	1.20	o	–	[2]
9.	*Berula erecta* (Huds.) Coville	1.20	o	o-m	[2], [8]
10.	*Bidens tripartita* Linn.	2.20	b	–	[2]
11.	*Butomus umbellatus* L.	1.90	o-a	–	[2]
12.	*Callitriche cophocarpa* Sendtn.	0.80	x-b	–	[2]
13.	*Callitriche hamulata* Kütz.	–	-	e	[8]
14.	*Callitriche hermaphroditica* L.	1.00	o	–	[2]
15.	*Callitriche obtusangulata* Le Gall	1.00	o	e	[2], [8]
16.	*Callitriche polymorpha* Lönnr.	0.80	x-b	–	[2]
17.	*Callitriche platycarpa* Kütz.	–	-	m	[8]
18.	*Carex acuta* L.	0.70	o-x	–	[2]
19.	*Carex gracilis* Curt.	0.70	o-x	–	[2]
20.	*Ceratophyllum demersum* L.	2.20	b	e	[2], [5], [8]
21.	*Cicuta virosa* L.	1.80	o-a	–	[2]
22.	*Coleogeton pectinatus* (L.) D. H. Les and Haynes	2.70	a-o	–	[2]
23.	*Eichhornia crassipes* (C. Mart.) Solms	1.90	o-a	–	[2]
24.	*Elatine hydropiper* L.	1.50	o-b	–	[2]
25.	*Eleocharis palustris* L.	1.10	o	–	[2]
26.	*Elodea Canadensis* Rich.	2.10	b	me	[2], [8]
27.	*Glyceria fluitans* (L.) R. Br.	1.50	o-b	e	[2], [5]
28.	*Glyceria maxima* (Hartm.) Holmb.	2.30	b	–	[2]
29.	*Groenlandia densa* (L.) Fourr.	–	–	me	[8]

Table 7b: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Magnoliophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Magnoliophyta				
30.	*Hippuris vulgaris* L.	2.20	b	–	[2]
31.	*Hottonia palustris* L.	1.70	b-o	–	[2]
32.	*Hydrocharis morsus-ranae* L.	2.10	b	–	[2]
33.	*Iris pseudocorus* L.	1.30	o	–	[2]
34.	*Juncus conglomeratus* L.	1.30	o	–	[2]
35.	*Lemna gibba* L.	2.40	b-a	–	[2]
36.	*Lemna minor* L.	2.20	b	–	[2]
37.	*Lemna polyrrhiza* L.	2.10	b	–	[2]
38.	*Lemna triscula* L.	1.90	o-a	–	[2]
39.	*Limnanthemum nymphoides* (L.) Hoffmanns. et Link	1.80	o-a	–	[2]
40.	*Limnanthemum peltatum* Griseb.	1.80	o-a	–	[2]
41.	*Limosella aquatica* L.	1.60	b-o	–	[2]
42.	*Malaxis paludosa* (L.) Swartz	1.90	o-a	–	[2]
43.	*Myriophyllum alterniflorum* D. C.	0.90	x-b	e	[2], [8]
44.	*Myriophyllum spicatum* L.	2.30	b	me	[2], [5]
45.	*Myriophyllum verticillatum* L.	1.80	o-a	e	[2], [5]
46.	*Najas marina* L.	1.90	o-a	e	[2], [5]
47.	*Nasturtium officinale* W. T.Aiton	–	–	e	[9]
48.	*Nuphar lutea* (L.) Smith	1.80	o-a	–	[2]
49.	*Nymphaea alba* L.	1.90	o-a	–	[2]
50.	*Nymphoides peltata* (S.G. Gmel.) O. Kuntze	1.80	o-a	–	[2]
51.	*Oenanthe aquatica* (L.) Poiret	1.50	o-b	–	[2]
52.	*Oenanthe fluviatilis* (Bab.) Coleman.	–	–	e	[8]
53.	*Oenanthe phellandrium* Lam.	1.50	o-b	–	[2]
54.	*Persicaria amphibian* (L.) Delarbre	2.10	b	–	[2]
55.	*Phalaris arundinacea* L.	2.10	b	–	[2]
56.	*Phalaroides arundinacea* (L.) Rauschert	2.10	b	–	[2]
57.	*Phellandrium aquaticum* L.	1.50	o-b	–	[2]
58.	*Phragmites australis* (Cav.) Steud.	2.30	b	me	[2], [5]

Table 7c: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Magnoliophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Magnoliophyta				
59.	*Phragmites communis* Trin.	2.30	b	–	[2]
60.	*Polygonum amphibium* L.	2.10	b	–	[2]
61.	*Polygonum amphibium* f. *natans* Moench	–	–	he	[5]
62.	*Potamogeton alpinus* Balbis	1.10	o	e	[2], [8]
63.	*Potamogeton berchtoldii* Fieber	1.90	o-a	–	[2]
64.	*Potamogeton coloratus* Hornem.	–	–	ot	[8]
65.	*Potamogeton crispus* L.	2.60	a-o	he	[2], [5]
66.	*Potamogeton gramineus* L.	1.50	o-b	–	[2]
67.	*Potamogeton lucens* L.	2.00	b	he	[2], [5], [8]
68.	*Potamogeton natans* L.	1.50	o-b	he	[2], [5]
69.	*Potamogeton nodosus* Poir.	–	–	he	[8]
70.	*Potamogeton pectinatus* L.	1.70	b	he	[2], [5], [8]
71.	*Potamogeton perfoliatus* L.	2.30	b	–	[2]
72.	*Potamogeton polygonifolius* A.Benn.	–	–	ot	[8]
73.	*Potamogeton pusillus* L.	1.90	o-a	he	[2], [5]
74.	*Ranunculus aquatilis* L.	2.20	b	–	[2]
75.	*Ranunculus fluitans* Lamk.	1.70	b-o	e	[2], [8]
76.	*Ranunculus hederaceus* L.	2.60	a-o	–	[2]
77.	*Ranunculus peltatus* Schrank	–	–	o-m	[8]
78.	*Sagittaria sagittifolia* L.	1.80	o-a	–	[2]
79.	*Schoenoplectus lacustris* (L.) Palla	2.20	b	he	[2], [5]
80.	*Scirpus lacustris* L.	2.20	b	–	[2]
81.	*Sium erectum* Hudson	1.20	o	–	[2]
82.	*Sium latifolium* L.	2.10	b	–	[2]
83.	*Sparganium erectum* L.	1.70	b-o	he	[2], [5]
84.	*Sparganium ramosum* Hudson	1.70	b-o	–	[2]
85.	*Spirodela polyrrhiza* (L.) Schleid.	2.10	b	–	[2]
86.	*Trapa natans* L.	2.00	b	–	[2]
87.	*Typha angustifolia* L.	1.10	o	me	[2], [5]

Table 7d: Index saprobity S, saprobic zone groups, and a group of trophic states for species of aquatic Magnoliophyta species with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Magnoliophyta				
88.	*Typha latifolia* L.	1.90	o-a	he	[2], [5]
89.	*Utricularia australis* R. Brown	2.00	b	–	[2]
90.	*Utricularia vulgaris* L.	2.10	b	–	[2]
91.	*Veronica anagallis-aquatica* L.	2.20	b	–	[2]
92.	*Veronica beccabunga* L.	1.00	o	–	[2]
93.	*Wolffia arrhiza* (L.) Wimmer	2.10	b	–	[2]
94.	*Zannichellia palustris* L. 1753	2.70	a-o	e	[2], [5], [8]

Table 8: Index saprobity S, saprobic zone groups, and group of trophic states for species of aquatic Polypodiophyta and Pteridophyta with numbered source data.

No.	**Species**	Index S	Saprobity group	Trophic state group	References
	Polypodiophyta				
1.	*Equisetum fluviatile* L.	1.10	o	–	[2]
2.	*Equisetum limosum* L.	1.10	o	–	[2]
3.	*Equisetum palustre* L.	1.00	o	–	[2]
4.	*Isoetes lacustirs* L.	0.30	x	–	[2]
5.	*Marsilea quadrifoliata* L.	1.00	o	–	[2]
6.	*Salvinia natans* (L.) All.	1.50	o-b	–	[2]
	Pteridophyta				
1.	*Azolla filiculoides* Lam.	1.00	o	–	[2]

Table 9a: Index saprobity S, saprobic zone groups, and group of trophic state for species of aquatic Charophyta with numbered of source data.

No	**Species**	Index S	Saprobity group	Trophic state group	References
	Charophyta				
1.	*Chara aculeolata* Kützing 1832	–	–	om	[3]
2.	*Chara aspera* Willdenow 1809	1.20	o	om	[2], [3]
3.	*Chara baltica* (Hartman) Bruzelius 1824	–	–	o-e	[3]
4.	*Chara baueri* A.Braun 1847	–	–	e	[6]
5.	*Chara braunii* C.C.Gmelin 1826	1.20	o	o-e	[2], [3]
6.	*Chara canescens* Loiseleur 1810	–	–	o-e	[3]
7.	*Chara connivens* Salzmann ex Braun A. 1835	–	–	om	[1], [3]
8.	*Chara contraria* Braun A. ex Kützing 1845	1.10	o	o-e	[2], [3]
9.	*Chara contraria* var. *excels* (Allen T. F.) Raam, 2010	–	–	m	[4]
10.	*Chara denudata* A.Braun, 1843	–	–	m	[1], [3], [7]
11.	*Chara filiformis* Hertzsch 1855	–	–	om	[3]
12.	*Chara globularis* Thuiller 1799	1.20	o	me	[2], [3], [5]
13.	*Chara hispida* Linnaeus 1753	0.90	x-b	he	[2], [3], [5]
14.	*Chara horrida* Wahlstedt 1862	–	–	me	[3]
15.	*Chara papillosa* Kützing 1834	–	–	om	[3]
16.	*Chara strigosa* A.Braun 1847	–	–	om	[3]
17.	*Chara subspinosa* Ruprecht 1846	–	–	om	[3]
18.	*Chara tenuispina* A.Braun 1835	1.10	–	om	[2], [3]
19.	*Chara tomentosa* Linnaeus 1753	1.20	–	o-e	[2], [3], [5]
20.	*Chara virgata* Kützing 1834	–	–	o-e	[3]
21.	*Chara vulgaris* Linnaeus 1753	1.10	–	o-e	[1], [2], [3]
22.	*Lamprothamnium papulosum* (Wallroth) J. Groves 1916	–	m	–	[3]
23.	*Lychnothamnus barbatus* (Meyen) Leonhardi 1863	–	–	ot	[3]
24.	*Nitella capillaries* (Krocker) J.Groves and Bullock-Webster 1920	1.50	o-b	om	[2], [3]
25.	*Nitella confervacea* (Brébisson) Braun A. ex Leonhardi 1863	0.80	x-b	om	[2], [3]
26.	*Nitella flexilis* (Linnaeus) Agardh C. 1824	1.30	o	om	[2], [3]
27.	*Nitella gracilis* (Smith J. E.) Agardh C. 1824	1.10	o	om	[2], [3]
28.	*Nitella hyalina* (De Candolle) Agardh C.	–	–	me	[3]

Table 9b: Index saprobity S, saprobic zone groups, and group of trophic state for species of aquatic Charophyta with numbered of source data.

No	**Species**	Index S	Saprobity group	Trophic state gr.	References
	Charophyta				
29.	*Nitella mucronata* (Braun A.) Miquel 1840	1.30	o	me	[1], [2], [3]
30.	*Nitella opaca* Agardh C. 1824	1.30	o	om	[2], [3]
31.	*Nitella syncarpa* (Thuillier) Chevallier 1827	1.30	o	om	[2], [3]
32.	*Nitella tenuissima* (Desvaux) Kützing 1843	0.80	x-b	o-e	[2], [3]
33.	*Nitella translucens* (Persoon) Agardh C. 1824	-	-	om	[3]
34.	*Nitellopsis obtuse* (Desvaux) Groves J. 1919	-	-	m	[3], [5]
35.	*Tolypella intricata* Leonhardi 1863	0.80	x-b	o-e	[2], [3]
36.	*Tolypella prolifera* Leonhardi 1863	1.10	-	me	[2], [3]
37.	*Tolypella glomerata* (Desvaux) Leonhardi 1863	-	-	om	[3]
38.	*Tolypella nidifica* (Müller O. F.) Braun A. 1857	-	-	om	[3]

The most prosperous indicator group was Magnoliophyta with 94 species, then followed Bryophyta with 59 indicators of trophic state and organic pollution, and Proteobacteria with 62 species (Tab. 10). These groups included more than 2/3 of the total indicators represented in tables 2-8. Because higher plants and mosses are widely studied and represented in water bodies, they, with about half of the species list (153 species-indicators), may be enough for bioindicational assessment of organic pollution and trophic state of water.

Table 10: Distribution of species-indicators of saprobity and trophic state over phyla of aquatic inhabitants.

No.	Phylum	No. of species
1.	Ascomycota	1
2.	Bigyra	19
3.	Bryophyta	59
4.	Charophyta	38
5.	Chlorobi	17
6.	Chloroflexi	15
7.	Katablepharidophyta	4
8.	Magnoliophyta	94
9.	Polypodiophyta	6
10.	Proteobacteria	62
11.	Pteridophyta	1
Total		316

As an example of the bioindicational assessment of some waterbodies, state, and level of organic pollution, two histograms were constructed on the base of tables 2-8. Figure 1 shows the distribution of species number in ecological groups placed on the x axes to increase organic pollution or trophic state. The Class of Water Quality histogram reveal two indicator groups divided into clusters of clear water and organically polluted water. In the clear-water group, the indicators of class 3 are the richest and followed class 2, this group combined species of macrophytes and mosses. Between the groups of polluted waters, class 6 prevailed, including bacteria and flagellated protozoan with the ability of heterotrophic nutrition.

The distribution of trophic state indicators also reveal two groups: prevailing oligo-mesotraphentic species of macrophytes and mosses, and the second one that included high-trophicity indicators of hypertrophic conditions, which mainly contain flagellates and bacteria. Therefore, collected data about organic pollution indicators with species-specific index S can improve the assessment results because Index S is related to about of hundred chemical and biological variables of aquatic ecosystems (Romanenko et al., 1990; Barinova, 2017b).

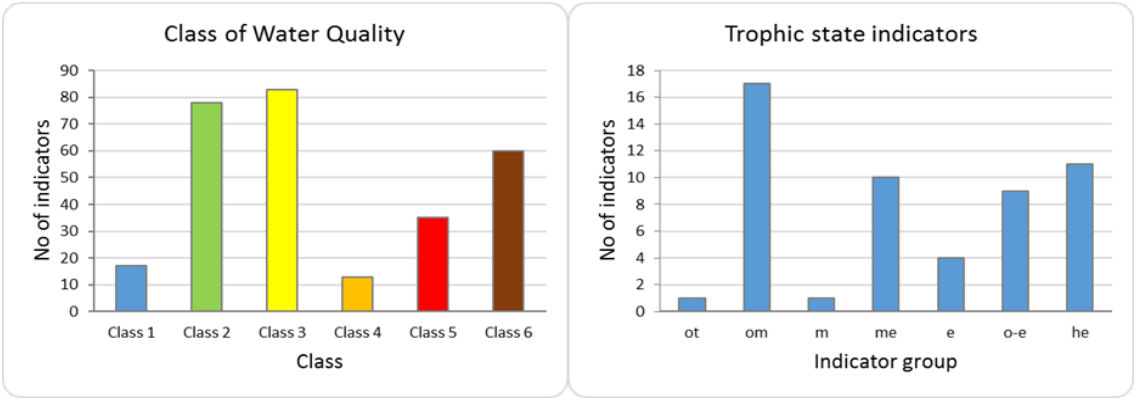

Figure 1: Distribution of species-indicators of organic pollution over Water Quality Classes and groups of trophic state conditions. Ecological groups are located on the x-axis following the increase of the indicated parameter. Class of water quality is as in EU colors.

CONCLUSIONS

To be able to determine the water quality by organic pollution and assess the aquatic ecosystem trophic state, we have collected the relevant ecological data from nine main references published as books, papers, or electronic resources for each of the aquatic species of macrophytes and some other aquatic inhabitants that are non-algae or cyanobacteria and non-invertebrates. Therefore, the list of indicators includes 316 species belonging to 11 phyla of aquatic macrophytes, mosses, charophytes, protozoan, and bacteria. In comparison, macrophytes and mosses prevail and demonstrate preferences of low to middle organically polluted waters. Classes 2 and 3 and oligo- to the mesotrophic environment, the indicator-species in protozoan and bacteria preferred organically polluted waters classes of 5-6 and high trophic conditions. Data collected of organic pollution indicators with species-specific index S can improve water quality and trophic state assessment to monitor organic pollution in diverse continental water bodies.

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A STUDY ON PHYTO-CHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF SPICES ON PATHOGENIC BACTERIA ISOLATED FROM COMMERCIALLY IMPORTANT EDIBLE MARINE FISHES, *EUTHYNNUS AFFINIS* (CANTOR), *KATSUWONUS PELAMIS* L. AND *AUXIS THAZARD* (LACEPEDE) (FAMILY SCOMBRIDAE)

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KEYWORDS: Tuna fishes, pathogenic bacteria, spices, phyto-chemicals.

ABSTRACT

Ten bacterial species were isolated and monthly variations in their count were recorded from three edible tuna fishes. Phytochemical analysis and antibacterial activity of hexane, chloroform, methanol, and distilled water extracts of twelve common spices, against the isolated bacteria were evaluated. The study indicates that these pathogenic bacteria in all three tuna fish species cause various human health problems upon consumption.

ZUSAMMENFASSUNG: Eine Studie zur phytochemischen Analyse und antibakteriellen Vorgängen bei Stämmen pathogener Bakterien isoliert aus kommerziell wichtigen, eßbaren Meeresfischen, *Euthynnus affinis* (Cantor), *Katsuwonus pelamis* L. und *Auxis Thazard* (Lacepede) (Family Scombridae).

Zehn Bakterienarten wurden isoliert und monatliche Variationen ihrer Anzahl bei drei essbaren Thunfischen, aufgezeichnet. Phytochemische Analysen und antibakterielle Aktivität von Hexan-, Chloroform-, Methanol- und destillierten Wasserextrakten von zwölf gängigen Gewürzen, wurden mit ihrer Wirkung auf die isolierten Bakterien bewertet. Die Studie zeigt, dass diese in allen drei Thunfischen art vorkommenden pathogenen Bakterien beim Menschen nach Verzehr verschiedene gesundheitliche Probleme verursachen.

REZUMAT: Un studiu privind analiza fitochimică și activitatea antibacteriană a condimentelor asupra bacteriilor patogene izolate din pești marini comestibili importanți din punct de vedere comercial, *Euthynnus affinis* (Cantor), *Katsuwonus pelamis* L. și *Auxis thazard* (Lacepede) (Familia Scombridae).

Zece specii bacteriene au fost izolate și s-au înregistrat lunar variațiile numărului lor de la trei pești de ton comestibili. Au fost evaluate analizele fitochimice și activitatea antibacteriană a extractelor cu hexan, cloroform, metanol și apă distilată din douăsprezece condimente comune, împotriva bacteriilor izolate. Studiul indică faptul că aceste bacterii patogene din cele trei specii de ton provoacă diverse probleme de sănătate umană în urma consumului.

INTRODUCTION

Fish is the primary source of animal protein available for humans for the maintenance of a healthy body in many parts of the world and this is especially true in most of the developing countries (Arannilewa et al., 2005; Del Monte-Luna, 2016). Fish is also a vitamin and mineral-rich food for young and old human consumers (Edem, 2009; Koffi-Nevry et al., 2011). The nutritional characteristics of fish and fishery products are of vital interest to human consumers. Polyunsaturated fatty acids from fish have been reported to have preventive and/or curative effects for several diseases including arterial hypertension (Turkmen et al., 2005), cancers, and inflammatory diseases (Marchioli, 2001). Even though, sea foods are nutritive, they act as a vehicle for the pathogenic bacteria naturally occurring in the aquatic environment referred to as indigenous or derived from the post-harvest contamination (Wallace et al., 1999; Gillespie et al., 2001). The microbial flora of freshly caught fish, is largely a reflection of microbial quality of the waters from where they are harvested. The microorganisms present in the environment enable them to enter the food chain through raw materials and are a major problem in convenience foods and mass catering (Beattie and William, 2000; Guinebretiere et al., 2006). The bacterial diseases are caused mainly due to contaminated water and sea foods (Musa et al., 2008). The majority of reported sea food-associated disease outbreaks are caused by toxins and bio-toxins (Chen et al., 2010).

There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at partial or total replacement of antimicrobial chemical additives. Gould (1995) has emphasized the possible use of spices and their derivatives as alternatives for inclusion in a new perspective of food conservation called “natural antimicrobial system”. The chief significance of the spices extract as an alternative to chemical preservatives is to minimize the side-effects of the latter and simultaneously improve the shelf-life of the food products. Natural products and naturally derived components from plants have applications in controlling pathogens in foods (Marina et al., 2013). The antioxidant and antibacterial property of added material is very important to improve the shelf life of food material and at the same time provide safety to consumers. The goal is to isolate, purify, stabilize, and include natural antioxidants and antimicrobials into foods without affecting sensory, nutritional, and safety characteristics (Suppakul et al., 2003; Sofia et al., 2019).

India plays a major role in the global seafood export among the Asian countries (Shyam et al., 2012). More than 50% of the marine fisheries production is accounted for by trawl fisheries. Among this, Andhra Pradesh contributed to 9% of Indian trawl landing of which a share of 51% is contributed to by Visakhapatnam Fishing Harbour (Sudharani, 2014). Most scombrids (tunas, mackerels, and bonitos) are important food and commercial export quality fishes. Much of the tuna catch is harvested for canning (Wheeler, 1985). Visakhapatnam fishing harbour holds bumper landings of scombrid fishes every year (Hanumantha et al., 2011). Tunas are among the largest and most specialized and commercially important of all fishes (Collette and Nauen, 1983).

With this background, the present study evaluate the antimicrobial properties of hexane, chloroform, methanol, and distilled water extracts of twelve common spices used as food additives *Coriander sativum* L., *Cuminum cyminum* L., *Trachyspermum ammi* (L.) Sprague ex Turrill, *Foeniculum vulgare* Mill. (Apiaceae), *Trigonella foenumgraecum* L. (Fabaceae), *Zingiber officinale* Roscoe, *Curcuma longa* L. (Zinziberaceae), *Allium sativum* L. (Amaryllidaceae), *Piper nigrum* L. (Piperaceae), *Cinnamomum zeylanicum* L. (Lauraceae), *Syzygium aromaticum* (L.) Merr. and Perry L. M. (Myrtaceae) and *Myristica fragrans* Houtt. (Myristicaceae) to control the growth of food-borne pathogenic bacteria, *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pyogenes* (Gram-positive)

Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Shigella sp., and *Vibrio cholerae* (Gram-negative) isolated from three tuna fish species, *Euthynnus affinis* (Kawakawa or Mackerel Tuna), *Katsuwonus pelamis* (Skipjack Tuna) and *Auxis thazard* (Frigate Tuna). The plant part used was the seed in the case of the first five spices, *P. nigrum* and *M. fragrans*; rhizome in the case of *Z. officinale, C. longa*; bulb/clove in the case of *A. sativum*; bark in the case of *C. zeylanicum*; and flower buds in the case of *S. aromaticum.* The study also recorded monthly variations in the count of these bacteria and carried out phyto-chemical analysis of spices for alkaloids, flavonoids, phenols, tannins, quinones, terpenoids, cardiac glycosides, proteins, carbohydrates, saponins, and steroids as they have an important role in expressing the anti-bacterial activity.

MATERIAL AND METHODS

Microbial analysis

Marine fish species, *Euthynnus affinis, Katsuwonus pelamis,* and *Auxis thazard* (Scombridae Family) (Figs. 1a-c) were collected during October 2018 – February 2019 from the landing centre at Visakhapatnam (17.68° N latitude and 83.21° E longitude), a largest coastal city in Andhra Pradesh State, situated on the East Coast of India. The fish samples were immediately transferred into an ice box, which were later brought to the laboratory for identification and microbial analysis. The samples were aseptically removed from the ice box and were placed on sterile trays with the aid of a sterile knife. Then, cuts were made from the edible parts of the fishes and from each fish one g were taken, homogenates were made in 10 mL of distilled water. The crushed samples were diluted from 10^{-1} to 10^{-3} for each fish sample. Microbial analysis is performed by adopting standard procedures (APHA, 1984, 1998).

Antimicrobial analysis

Twelve spices were purchased from the local market of Visakhapatnam for analysis. Collected samples were ground into a fine powder and paste (dried seeds, flower buds, fresh cloves, fresh, and dried rhizome, and bark) and measured about 200 g for the process. The solvent extraction was carried out with four solvents namely hexane (non-polar and 99% assay) and three polar solvents: chloroform (99.5% assay), methanol (99.5% assay), and distilled water (100%) using Soxhlet extraction process. The Soxhlet apparatus used was of 500 mL capacity. The Whatman No. 1 filter paper was placed into the thimble and added 150 g of the sample material for each experiment. The purpose of using Whatman No. 1 filter paper was to ensure the presence of samples inside the thimble during the experiment. About 500 mL of extraction solvent was then poured into the round bottom extraction flask and placed on the heating mantle. The extraction process was carried out for four-six hours with each batch at a temperature not exceeding the boiling point of the solvent. These extracts were distilled by the distillation unit and were transferred to amber glass bottles and kept at 4°C until examined. Extracts were dissolved in Dimethyl sulphoxide (DMSO) solution to prepare different concentrations (50 µL, 100 µL, 150 µL, and 200 µL). These extracts were tested against bacterial species isolated from tuna fish samples. Mueller Hinton Agar (Himedia) was used as a test medium for antimicrobial susceptibility. A petri dish containing Mueller Hinton Agar medium surface was spread with inoculum using a sterile swab. Using a sterile cork borer, nearly eight mm diameter wells were bored in the inoculated agar plates and different concentrations of extracts (50 µL, 100 µL, 150 µL, and 200 µL) were added into the wells. All the plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the zone of growth inhibition around the well by using agar well diffusion assay technique. The zone of inhibition of bacterial growth was measured by HiMedia's antibiotic zone scale.

Minimum inhibitory concentration (MIC)

Two mL of nutrient broth was dispensed in tubes and 100 µL of 24 h fresh culture was inoculated. Then 50-200 µL of different concentrations of extract was added to each tube. Each experiment was carried out in a triplicate set. Growth control was run parallelly with every experiment. All the experimental tubes were incubated for 24-48 h at 37°C. After the incubation period, turbidity was measured at 600 nm using a colorimeter. The 20% inhibition of the growth of test bacterial species was treated as Minimum Concentration Extract (MIC). The percentage of bacterial inhibition by each extract was calculated using the below equation.

$$\text{Percentage inhibition} = \frac{\text{Optical Density in control} - \text{Optical Density in test} \times 100}{\text{Optical Density in control}}$$

Phytochemical analysis of spices

The qualitative analysis of methanol extract of all 12 spices was carried out for alkaloids, flavonoids, saponins, tannins, phenols, proteins, cardiac glycosides, terpenoids, carbohydrates, quinines, and steroids by using standard protocols. To one mL of extract, two mL of concentrated sulphuric acid and few drops of 40% formaldehyde (one mL of Marquis Reagent) were added and mixed; appearance of dark orange or purple colour was taken as the presence of alkaloids. To two mL of extract, a few drops of 20% sodium hydroxide were added; formation of intense yellow colour was observed. To this, a few drops of 70% dilute hydrochloric acid were added and yellow colour disappeared. Formation and disappearance of yellow colour was taken as the presence of flavonoids. To two mL of extract, six mL of distilled water was added and shaken vigorously; formation of bubbles or persistent foam was taken as the presence of saponins. To two mL of extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour was taken as the presence of tannins. To two mL of extract, two mL of 5% aqueous ferric chloride was added; formation of blue colour was taken as the presence of phenols. To two mL of extract, one mL of 40% sodium hydroxide and a few drops of 1% copper sulphate were added; formation of violet colour was taken as the presence of peptide linkage molecules constituting proteins. To one mL of extract, 0.5 mL of glacial acetic acid and three drops of 1% aqueous ferric chloride solution were added; formation of a brown ring at the interface was taken as the presence of cardiac glycosides. To one mL of extract, 0.5 mL of chloroform and a few drops of concentrated sulphuric acid were added; formation of reddish brown precipitate was taken as the presence of terpenoids. To one mL of extract, a few drops of Molisch's reagent and one mL of concentrated sulphuric acid at the side of the tubes was added. The mixture was then allowed to stand for two to three minutes; formation of red or dull violet colour was taken as the presence of carbohydrates. To two mL of extract, one mL of 5% chloroform, and one mL of 10% ammonia solution were added; formation of pink violet colour was taken as the presence of quinines. To two mL of extract, two mL of acetic anhydride and two mL of sulphuric acid were added; the colour change from violet to green colour was taken as the presence of steroids.

Figure 1a: *Euthynnus affinis* (Cantor, 1849).

Figure 1b: *Katswonus pelamis* (Linnaeus, 1978).

Figure 1c: *Auxis thazard* (Lecepede, 1800).

RESULTS AND DISCUSSION

Microbiological quality of fish

Microbiological quality and safety of freshly caught fish depends on the hygienic quality of the aquatic environment. Fish are susceptible to contamination, especially those from freshwater environments due to slow water exchange and high anthropogenic contamination (Orban et al., 2007). In aquatic environments, microorganisms spread easily between habitats and hosts. In this process, fish may obtain microorganisms from water, sediment, and food organisms indicating that fish are affected by their feeding behaviour, food availability in the environment and pollution in the water (Geldreich and Clarke, 1966; Olafsen, 2001). Even though sea foods are nutritive, they act as a vehicle for the pathogenic bacteria naturally occurring in the aquatic environment (Al-Sheraa, 2018). Therefore, the quality of seafood depends on the quality of water where the fishes are caught and the sanitary conditions at the landing centre. In this study, the microbiological assessment for *Ethynnus affinis, Katsuwonus pelamis*, and *Auxis thazard*. Comparative analysis of these fish samples were made and the microbial flora present in these samples were isolated by using Most Probable Number (MPN) test, Total Viable Count (TVC), Total Plate Count (TPC), and Total Coliform Count (TCC). The bacterial isolates were identified by standard biochemical tests.

Most probable number (MPN) method

Most Probable Number is a method used to estimate the concentration of viable microorganisms. A group of bacteria commonly referred to as faecal coliforms act as an indicator of faecal contamination of water and food. The coliform bacteria are aerobic and facultative anaerobic, gram negative, non-spore forming, rod-shaped bacteria that ferment lactose with gas and acid production in 24 to 48 h at 35°C. The presence of the coliform group of bacteria, mainly *Enterobacter, Escherichia*, and *Klebsiella* in fish presents a health hazard to humans. The microbial quality of the fish indicate that all tissue samples except muscle parts were contaminated with the high numbers of faecal coliform *E. coli* and hence it is the most common contaminant indicating the faecal pollution of sea water.

In this study, the monthly total coliform count in MPN method for all the three fresh tuna fish samples are presented in table 1. In *E. affinis*, the total coliform count ranged from four to 20 MPN/g with a mean of 9.27 ± 5.75. The highest count of 20 MPN/g was recorded in November 2018 and September 2019 and the lowest count of four MPN/g in March 2019. In *K. pelamis*, total coliform count ranged from 6.5 to 45 MPN/g with a mean of 16.45 ± 10.48. The maximum count of 45 MPN/g was recorded in September 2019 and lowest count of 6.5 MPN/g in March 2018. In *A. thazard*, the total coliform count ranged from 7.5 to 25 MPN/g with mean value of 15.45 ± 6.49. The maximum count 25 MPN/g was recorded in March and April 2019 and the lowest count of 7.5 MPN/g in November 2018 and September 2019.

The faecal coliform bacteria were found in all three fish samples. The MPN values of all the three fishes indicated that *K. pelamis* has the highest TFC (40% value). High counts of faecal coliform and the prevalence of pathogenic bacteria in this species indicate that the water environment from where it was captured is contaminated. The results obtained in this study are similar to the range of the coliform group (*E. coli*) reported by different workers (Geetha et al., 2009; Begum et al., 2010; Koteswara Rao et al., 2017). These authors attributed the high count of faecal coliform to the contamination of samples before or during handling, processing and marketing. Further, they also mentioned that *E. coli* can survive long periods in tropical waters and once introduced into the water environment it becomes indigenous to that environment.

According to the USFDA standards, the fish samples tested for microbial analysis exceeded the permissible limits, but it is not an indicator of contamination of food but an indication of a variation in food quality and potential for pathogen survival and growth.

Table 1: Total coliform count in MPN Method.

Month, year	Name of the fish species		
	Ethynnus affinis	*Katsuwonus pelamis*	*Auxis thazard*
October 2018	11.5	20	20
November 2018	20	20	25
December 2018	4.5	9.5	9.5
January 2019	6.5	15	15
February 2019	6.5	11.5	9.5
March 2019	4	6.5	7.5
April 2019	4.5	7.5	7.5
May 2019	–	–	–
June 2019	7.5	15	15
July 2019	7.5	16	20
August 2019	9.5	15	16
September 2019	20	45	25
Mean and Std. Dev.	9.27 ± 5.75	16.45 ± 10.48	15.45 ± 6.49

Total viable count (TVC): Determination of the Total Viable Count (TVC) in a food product is one of the simplest and widely used microbiological techniques. TVC typically is conducted to obtain information about the microbiological quality of foods. It gives a quantitative estimate of the concentration of microorganisms such as bacteria, yeast or mould spores in a sample. The count represents the number of Colony Forming Units (CFU) per/g or per/mL of the sample. TVC analysis showed variation from species to species. The highest percentage (36%) of TVC was observed in *E. affinis* followed by 32% of TVC each by *K. pelamis* and *A. thazard*. The bacterial flora in freshly caught fish depends on the environment in which it is caught rather than on the fish species (Shewan, 1961). The detection of the bacterial load on the fish surface gives an idea about the quality of samples. When TVC reaches to 10^6/g or more in processed food or food products, then the food is considered spoiled (Shewan, 1970).

Total plate count (TPC): A number of aerobic and facultative anaerobic bacteria can be isolated by using Heterotrophic Plate Count. It includes both gram-positive and gram-negative bacteria. The TPC gives the total bacterial count present in one g of fish sample. They form colony forming units by counting this CFU/g. In this study, the number of microorganism/g was encountered. TPC mean values ranged from $2.67 \pm 0.70 \times 10^5$ CFU/g to $3.03 \pm 0.52 \times 10^5$ CFU/g. The lowest mean value was observed in *A. thazard* while the highest value was observed in *E. affinis* (Tab. 2). In *E. affinis,* the highest count of 3.54×10^5 CFU/g was observed in July 2019 and the lowest count of 1.92×10^5 CFU/g was observed in November 2018. In *K. pelamis*, the highest count of 3.81×10^5 CFU/g was observed in February 2019 and the lowest count of 1.7×10^5CFU/g in April 2019. In *A. thazard*, the highest count of 3.78×10^5 CFU/g was observed in October 2018 and the lowest count of 1.28×10^5 CFU/g in April 2019.

Table 2: Total plate count in CFU/gm (TPC).

Month	*Ethynnus affinis*	*Katsuwonus pelamis*	*Auxis thazard*
October 2018	3.16	3.22	3.78
November 2018	1.92	2.16	2.52
December 2018	2.2	2.04	2.04
January 2019	3.2	2.5	2.92
February 2019	2.92	3.81	3.16
March 2019	3.1	2.06	2.0
April 2019	3.15	1.7	1.28
May 2019	–	–	–
June 2019	3.16	3.22	2.52
July 2019	3.54	3.62	3.22
August 2019	3.52	3.05	3.1
September 2019	3.5	2.6	2.8
Mean and Std. Dev.	3.03 ± 0.52	2.73 ± 0.70	2.67 ± 0.70

Magbooljan and Revathi (2014) reported that the bacterial diversity in the digestive tract of the fish generally varies due to the hydro-biological fluctuations occurring in the natural systems. It is considered that the bacterial ecology of fishes is connected to environmental factors such as water pollution, hygienic procedures of slaughter, handling, transport, commercialization, and storage conditions. Janina et al. (2011) reported that the microflora of digestive tracts of aquatic animals is the first to be affected by any pollutants appearing in the water. The abundance of bacteria in aquatic organisms depends on fish species, nutrition habits, as well as seasonal and environmental effects, internal and external factors (Spanggaard et al., 2000; Austin, 2002; Ringo et al., 2008). The bacterial diversity in the fish may also increase with the increase of water temperature (Hossain et al., 1999).

Characterization and identification of bacterial species

In this study, a total of 16 bacterial species were isolated from the fish samples. The isolated colonies were taken based upon the morphological characteristics on Nutrient Agar (NA) and different selective medias. These isolated bacterial species were predominant by gram negative bacteria. Out of 16, 10 bacterial species were identified by using standard biochemical tests shown in tables 3 and 4. The remaining six bacterial species belong to the same genera. The identified isolates include *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Enterococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp., *Vibrio* sp., *Salmonella* sp., and *Shigella* sp. The isolated bacterial species were identified by using standard microscopic and macroscopic techniques that are Gram staining followed by biochemical tests – Indole, Methyl red, Voges Proskauer, Citrate utilization (IMVC tests), Nitrate reduction, Hydrogen Sulphide production, Urease test, Catalase test, Oxidase test, Motility, Starch hydrolysis, Gelatin liquefaction, Fermentation of Carbohydrates (acid and gas production) (Cappuccino, 1999). The genera and species present in the samples were identified according to their characteristics as outlined in Bergey's manual of Systematic Bacteriology (David et al., 2001).

Table 3: Morphological characteristics of isolated microorganisms.

Morphological characteristics	Organism
Non-spore forming and non-motile, gram positive cocci, circular, low convex with entire margin, smooth, medium, opaque colony on Nutrient Agar, yellow colour colonies on Mannitol Salt Agra Media grown at pH 7 and 37°C.	*Staphylococcus* sp.
Gram positive cocci, thin, even, growth on Nutrient Agar, black or brown colour colonies on Bile esculin Agar.	Group D *Streptococcus* sp.
Gram positive rod, spore forming, abundant, opaque, white waxy growth on Nutrient Agar.	*Bacillus* sp.
Gram positive cocci, circular, smooth and cream or white colour colonies with entire edges on Nutrient Agar, magenta red colour colonies on MacConkey Agar.	*Enterococcus* sp.
Gram negative rod, circular, low convex, with entire margin, mucoid, opaque, growth on Nutrient Agar, green metallic sheen colony on Eosin Methylene Blue (EMB) Agar.	*Escherichia coli*
Gram negative rod, slimy, white somewhat translucent, raised growth on Nutrient Agar, dark pink colour colonies on MacConkey Agar.	*Klebsiella* sp.
Gram negative rod, abundant, thin, white medium turns green on Nutrient Agar. Pink colour colonies on Phenothalin diphospate Agar.	*Pseudomonas aeruginosa*
Gram negative curved rod, abundant, thick, mucous white colour colonies on Nutrient Agar. Yellow colour colonies on TCBS agar.	*Vibrio cholerae*
Gram negative curved rod abundant, thick, mucous white colour colonies on Nutrient Agar. Green colour colonies on TCBS agar.	*Vibrio parahaemolyticus*
Gram negative rod, thin even greyish growth on Nutrient Agar.	*Salmonella* sp.
Gram negative rod, thin even greyish growth on Nutrient Agar.	*Shigella* sp.

The microbial communities isolated from the fish samples during the study period showed a highly diverse and varied microbial population. Contamination of seafood with bacterial pathogens at source (in the sea) primarily arises from the marine environment; when seafood is consumed in large enough numbers it causes illness in humans. Some species of the bacteria possibly cause gastro-enteritis in humans and these bacteria may also be present naturally in the marine or more especially in the estuarine environment.

The percentage of bacterial population in three fish samples ranged from 7% to 11%. The individual percentages of isolated bacterial species were *B. cereus* (9-10%), *E. faecalis* (9-10%), *S. aureus* (11%), *S. pyogenes* (10-11%), *E. coli* (9-10%), *K. pneumoniae* (8-9%), *P. aeruginosa* 9%, *S. typhi* 8%, *Shigella* spp. 8%, *V. cholerae* 7% and unknown organisms (8-11%). The study indicated that the percentage of bacterial species varied in the different months in each fish species; the highest percentage was represented by *Staphylococci* and *Streptococci*. The presence of *S. aureus* indicates the contamination of the fish and its natural environment by human beings and warm blooded animals. Clucas and Ward (1996) recorded the presence of *S. aureus* in natural micro-flora of fish and shellfish in the U.K., Herrera et al. (2006) reported that the raw fish contamination with food borne bacterial pathogens occurs during post-harvest handling and processing procedures in Spain. In this study, *S. aureus* is the major causative agent of food poisoning in humans, as it is known to release entero-toxins causing severe illness in the gastro-intestinal tract. *E. coli, E. faecalis, P. aeruginosa*, and *B. cereus* showed moderate counts during different months while *K. pneumoniae, S. typhi, Shigella* spp., and *V. cholerae* showed low counts in all the three fish samples. The study indicates the presence of bacterial pathogens can be attributed to the contamination of the process, improper handling, hygienic, and sanitary conditions of Visakhapatnam Harbour. Drinking faecal contaminated water can also lead to an outbreak of the health issues due to these bacterial pathogens (Pelczar et al., 1993). *E. coli* and *Salmonella* spp. can survive for very long periods in tropical waters and once introduced may become adaptable to the new conditions favouring the growth of microorganisms in the environment and subsequently contaminating the fish species (Fujioka et al., 1988). In this context, the present study shows that the microbial quality of fish samples is not good due to the presence of *Vibrio* spp., *E. coli* counts in all three fish samples is less in the MPN test. *E. coli* and *Klebsiella* sp. are gram-negative bacteria and members of the normal intestinal flora of humans and animals and hence can be isolated from a variety of environmental sources. *E. coli* is associated with enteric infections. *B. cereus* is a toxin-producing facultative anaerobic gram-positive bacterium and commonly found in soil, vegetation, and food. It has the ability to quickly multiply at room temperature. It causes intestinal illnesses, leading to diarrhoea and nausea/vomiting. Specifically, the diarrheal illness is often related to meat, milk, vegetables, and fish, and hence *B. cereus* is commonly associated with food-borne illness (Omer et al., 2018; Kimura and Yokoyama, 2019).

P. aeruginosa is very important because this bacterium plays a considerable role as a potential pathogenic bacterium for humans and as an indicator of food quality as a spoilage organism. In aquaculture, *P. aeruginosa* and *P. fluorescens* have been considered as opportunistic pathogenic species (Altinok et al., 2006). In this study, *P. aeruginosa* was isolated from the all the fish samples. It is primarily a nosocomial pathogen and commonly present in soil, water, and vegetation. Its presence in the fish samples indicates the poor quality of all fish species; its presence is attributable to poor handling, improper storage system, and sanitary conditions at each step in the fish processing and selling.

Table 4: Biochemical characteristics of bacterial species.

Biochemical test	Bacterial species (A = Acid production only; AG: Acid + Gas production; ± = Variable reaction; + Positive; – = Negative; (+) = Late Positive; Negative = N; Positive = P; Variable = V; Fermentative = F; Facultative anaerobes = Fa; Oxidative = O; Non-fermentative = Nf.									
	Bacillus cereus	*Enterococcus faecalis*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella* spp.	*Vibrio cholerae*
Gram stain	P	P	P	P	N	N	N	N	N	N
Oxidative/ Fermentative	–	F	F	Fa	F	F	O	–	Nf	–
Gas	–	–	N	–	P	P	P	N	P	N
Catalase	P	N	P	N	P	P	P	P	P	P
Oxidase	N	N	N	N	N	N	P	N	N	P
Indole	N	N	N	N	P	N	N	N	V	P
MR	N	–	P	P	P	N	N	P	P	N
VP	P	P	P	N	N	P	N	N	N	V
Citrate	P	N	P	–	N	P	P	N	N	P
Urease	–	N	P	N	N	P	N	N	N	N
H_2S	–	N	N	–	N	N	N	P	N	N
Nitrate reduction	V	P	P	–	P	P	P	P	P	P
Gelatin	N	V	P	–	N	N	P	N	N	P
Starch hydrolysis	P	N	N	N	N	N	N	N	N	N
Lactose fermentation	–	A	–	A	AG	AG	–	–	–	AG
Glucose fermentation	A	A	A	A	AG	AG	–	AG	A	AG
Sucrose fermentation	A	A	A	A	A (+)	AG	–	AG	A (±)	AG

Phyto-chemical analysis of spices

Secondary metabolites produced by microorganisms exhibit various types of biological activities. Plants accumulate a variety of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, quinones, terpenoids, cardiac glycosides, proteins, carbohydrates, saponins, and steroids. The spices examined in this study include *C. sativum* (S1), *T. ammi* (S2), *C. cyminum* (S3), *F. vulgare* (S4), *M. fragrans* (S5), *T. foenum-graecum* (S6), *P. nigrum* (S7), *S. aromaticum* (S8), *C. zeylanicum* (S9), *A. sativum* (S10), *Z. officinale* (S11), and *C. longa* (S12). The secondary metabolites recorded in these spices have been presented in table 5.

Table 5: Phyto-chemical analysis of twelve spices.

Test Name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	±	+
Tannins	+	–	–	+	+	+	+	+	+	–	+	+
Quinones	–	+	+	+	+	+	–	–	+	+	–	+
Terpenoids	+	–	+	+	+	+	-	+	+	+	+	+
Cardiac glycosides	+	+	–	+	+	+	+	+	+	+	+	+
Proteins	+	+	–	–	+	–	+	+	–	+	+	–
Carbohydrates	+	+	+	+	+	+	+	+	-	+	+	+
Saponins	+	+	–	+	+	+	+	+	+	+	–	+
Steroids	–	–	+	+	+	+	–	+	+	+	–	+

(+) = present; (–) = absent; *Coriandrum sativum* (S1), *Trachyspermum ammi* (S2), *Cuminum cyminum* (S3), *Foeniculum vulgare* (S4), *Myristica fragrans* (S5), *Trigonella foenum-graecum* (S6), *Piper nigrum* (S7), *Syzygium aromaticum* (S8), *Cinnamomum zeylanicum* (S9), *Allium sativum* (S10), *Zingiber officinale* (S11), and *Curcuma longa* (S12).

Alkaloids

Alkaloids are present in all twelve spices. The alkaloids contain heterocyclic nitrogen and are synthesized from amino acids or their intermediate derivatives (Brondz et al., 2007). Alkaloids cure many deadly human diseases (Bhattacharyya et al., 2007). They regulate Na+ ions and channels, microbial activity, have stimulating properties, and induce immunogenic cell death. They are also known to have inhibitory effects on angiogenesis and hence are useful in inhibiting the growth of cancerous cells (Aniszewski, 2015).

Flavonoids

Parajuli et al. (2012) reported that flavonoids are one of the most diverse group of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, anti-allergenic, antiviral, anti-inflammatory, and vaso-dilation actions. In this study, all twelve species of spices contain flavonoids and hence are valuable for humans.

Phenols

Pereira et al. (2009) reported that plant parts contain phenol compounds and they are excellent antioxidants which prevent oxidative damage related to ageing and diseases, such as atherosclerosis, diabetes, cancer, and cirrhosis. In this study, all spice species except *Z. officinale* indicated the presence of phenols; the *Z. officinale* sample showed different responses, the fresh ginger paste solvent extract was positive for phenols while the powder solvent extract was negative for phenols.

Tannins

Okwu and Okwu (2004) reported that tannins exhibit astringent properties and have wound healing properties. Chung et al. (1998) reported that tannins have traditionally been considered anti-nutritional, but his study showed that the anti-nutritional properties depend upon their chemical structure and dosage. Tannins inhibit bacterial infections in the urinary tract and have antibacterial, anti-enzymatic, antioxidant, anti-mutagenic, and anti-tussive properties. They are used in treating diarrhoea, ulcers, toothache, and for stopping bleeding. In this study, the test showed that tannins are absent in *T. ammi*, *C. cyminum*, and *A. sativum* while they are present in all other spice species. Therefore, the spice species indicated the present of tannins are valuable in treating certain human ailments.

Quinones

Liu (2011) reported that quinones are of pharmacological interest. They form a major class of cytotoxins and used in the fight against cancers. Some of them show anti-tumor activity. Further, they are useful as purgative and to treat cardiovascular diseases and have anti-microbial, anti-parasitic, and anti-tumor properties. In this study, quinones are absent in *C. sativum, P. nigrum, S. aromaticum,* and *Z. officinale* while they are present in all other eight species. Therefore, the spice species in which quinones are present are useful for treating certain human diseases.

Terpenoids

Terpenoids in herbs and spices are often used and are still used today to preserve food due to their microbicidal and insecticidal properties. Plant terpenoids play a role in traditional herbal remedies and contribute to scent production (Tassou et al., 2012). In this study, terpenoids are absent in *T. ammi* and *P. nigrum* while they are present in all other ten spice species. The spices in which the terpenoids are present can be exploited for use in traditional medicine and in food preservation.

Cardiac glycosides

Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis which causes the sugar part to be broken off, making the chemicals available for use. Many plant glycosides are used as medications. Their activity affects the gastrointestinal tract; cyanogenic glycosides interfere with iodine organification and can cause or promote goitre and hypothyroidism (Brito-Arias, 2007). In this study, cardiac glycosides are absent in only one spice species, *C. cyminum* while they are present in all other eleven spice species and hence the latter can be exploited for use in medications.

Proteins and carbohydrates

Proteins are essential macronutrients but not all food sources of protein are created equal. In this study, proteins are absent in *C. cyminum*, *F. vulgare, T. foenum-graecum, C. zeylanicum,* and *C. longa*. In all other seven spice species, proteins are present. Carbohydrates are one of the three main nutrients found in foods. The other two main nutrients are proteins and fats. Carbohydrates representing sugars, starches, and dietary fiber are the main source of energy for the body and they occur in plant foods. In this study, carbohydrates are absent in *C. zeylanicum* but they are present in all other eleven spice species. Therefore, the spices that contain proteins and carbohydrates can be used as food additives.

Saponins

Phyto-constituents such as saponins, phenolic compounds, and glycosides inhibit bacterial growth and protect plants against bacterial infections (Okwute, 1992). In this study, saponins are absent in *C. cyminum* and *Z. officinale* while they are present in all other ten spice species. Therefore, these ten spices can be evaluated for their inhibitory role against bacterial growth and infections.

Steroids

Patel and Savjani (2015) reported that plant steroids possess medicinal and pharmaceutical activities such as anti-tumor, immunosuppressive, hepato-protective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic, and cardiotonics. In this study, steroids are absent in *C. sativum, T. ammi, P. nigrum,* and *Z. officinale*. But, they are present in all other eight spice species and hence they can be considered for treating certain health problems.

Antimicrobial activity

A variety of plant- and spice-based antimicrobials are used by the food industry as natural agents to reduce or eliminate pathogenic bacteria and increase the overall quality of food products and extend the shelf-life of foods. In the present study, antibacterial activity of 50, 100, 150, and 200 µL/mL of hexane, chloroform, methanol and aqueous extracts of all twelve spice species were tested against pathogenic bacteria namely, *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella* spp., and *Vibrio cholerae* isolated from the samples of all the three fish species. The results obtained are shown in tables 6-18. The extracts of all twelve spices showed a varying degree of inhibition on each tested pathogenic bacterium (Figs. 2-5). The zone of inhibition ranged from 8 ± 0.3 to 35 ± 1.5 mm. The highest zone of inhibition (35 mm) was observed in 200 µL/mL concentration of each form of extract. The results indicated that the chloroform and aqueous water extracts of *C. zeylanicum* showed the maximum zone of inhibition at 200 µL/mL concentration against *E. coli* while methanol and aqueous extracts of *C. sativum* showed the minimum zone of inhibition (8 mm) at 50 µL/mL concentration against *S. aureus* and *S. pyogenes*.

Table 6: Zone of inhibition of Erythromycin (standard antibiotic).

Zone of inhibition in millimeters (mm)	
Microorganisms	Erythromycin (standard antibiotic)
Bacillus cereus	29 mm
Enterococcus faecalis	Nil
Staphylococcus aureus	16 mm
Streptococcus pyogenes	29 mm
Escherichia coli	14 mm
Klebsiella pneumonia	19 mm
Pseudomonas aeruginosa	18 mm
Salmonella typhi	15 mm
Shigella spp.	18 mm
Vibrio cholera	36 mm

Table 7: Result of Hexane extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Coriandrum sativum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	12 ± 0.5	12 ± 0.5	12 ± 0.5	12 ± 0.5
Staphyloccus aureus	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Streptococcus pyogenes	16 ± 0.7	17 ± 0.7	17 ± 0.7	18 ± 0.8
Escherichia coli	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	17 ± 0.7	18 ± 0.8	18 ± 0.8	18 ± 0.8
Pseudomonas aeruginosa	14 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Salmonella typhi	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Shigella spp.	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Vibrio cholerae	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Bacterial species	*Trachyspermum ammi*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	16 ± 0.7	16 ± 0.7	18 ± 0.8	22 ± 0.9
Enterococcus faecalis	15 ± 0.6	15 ± 0.6	17 ± 0.7	18 ± 0.8
Staphyloccus aureus	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Streptococcus pyogenes	17 ± 0.7	18 ± 0.8	19 ± 0.8	21 ± 0.9
Escherichia coli	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Klebsiella pneumonia	20 ± 0.9	25 ± 1.1	27 ± 1.2	27 ± 1.2
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Salmonella typhi	14 ± 0.6	14 ± 0.6	16 ± 0.7	17 ± 0.7
Shigella spp.	15 ± 0.6	15 ± 0.6	15 ± 0.6	17 ± 0.7
Vibrio cholerae	16 ± 0.7	17 ± 0.7	17 ± 0.7	19 ± 0.8
Bacterial species	*Cuminum cyminum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Enterococcus faecalis	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Staphyloccus aureus	10 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Streptococcus pyogenes	18 ± 0.8	18 ± 0.8	18 ± 0.8	18 ± 0.8
Escherichia coli	11 ± 0.4	12 ± 0.5	12 ± 0.5	14 ± 0.6
Klebsiella pneumonia	15 ± 0.6	16 ± 0.7	16 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Salmonella typhi	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Shigella spp.	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Vibrio cholerae	26 ± 1.1	26 ± 1.1	26 ± 1.1	27 ± 1.2
Bacterial species	*Foeniculum vulgare*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	17 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Staphyloccus aureus	10 ± 0.4	10 ± 0.4	11 ± 0.4	12 ± 0.5
Streptococcus pyogenes	19 ± 0.8	20 ± 0.9	21 ± 0.9	22 ± 0.9
Escherichia coli	14 ± 0.6	16 ± 0.7	17 ± 0.7	19 ± 0.8
Klebsiella pneumonia	15 ± 0.6	17 ± 0.7	17 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6
Salmonella typhi	13 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Shigella spp.	15 ± 0.6	17 ± 0.7	18 ± 0.8	19 ± 0.8
Vibrio cholerae	19 ± 0.8	19 ± 0.8	19 ± 0.8	20 ± 0.9

Table 8: Results of Hexane extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Myristica fragrans			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	14 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Staphyloccus aureus	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Streptococcus pyogenes	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Escherichia coli	15 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	Nil	11 ± 0.4	12 ± 0.5	13 ± 0.5
Shigella spp.	13 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6
Vibrio cholerae	11 ± 0.4	12 ± 0.5	13 ± 0.5	14 ± 0.6
Bacterial species	*Trigonella foenum-graecum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Staphyloccus aureus	Nil	11 ± 0.4	13 ± 0.5	15 ± 0.6
Streptococcus pyogenes	11 ± 0.4	12 ± 0.5	12 ± 0.5	13 ± 0.5
Escherichia coli	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Klebsiella pneumonia	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Salmonella typhi	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Shigella spp.	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	12 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6
Bacterial species	*Piper nigrum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	13 ± 0.5	14 ± 0.6	14 ± 0.6
Enterococcus faecalis	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Staphyloccus aureus	13 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6
Streptococcus pyogenes	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Escherichia coli	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	11 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Salmonella typhi	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Shigella spp.	12 ± 0.5	12 ± 0.5	12 ± 0.5	13 ± 0.5
Vibrio cholerae	15 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Bacterial species	*Syzygium aromaticum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	17 ± 0.7	17 ± 0.7	18 ± 0.8	19 ± 0.8
Enterococcus faecalis	11 ± 0.4	11 ± 0.4	12 ± 0.5	14 ± 0.6
Staphyloccus aureus	12 ± 0.5	12 ± 0.5	12 ± 0.5	12 ± 0.5
Streptococcus pyogenes	14 ± 0.6	17 ± 0.7	17 ± 0.7	18 ± 0.8
Escherichia coli	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	21 ± 0.9	23 ± 1.0	24 ± 1.0	24 ± 1.0
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Salmonella typhi	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Shigella spp.	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Vibrio cholerae	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6

Table 9: Result of Hexane extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Cinnamomum zeylanicum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	16 ± 0.7	16 ± 0.7	18 ± 0.8	19 ± 0.8
Enterococcus faecalis	17 ± 0.7	17 ± 0.7	19 ± 0.8	19 ± 0.8
Staphyloccus aureus	Nil	Nil	Nil	Nil
Streptococcus pyogenes	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Escherichia coli	30 ± 1.3	31 ± 1.4	31 ± 1.4	32 ± 1.4
Klebsiella pneumonia	13 ± 0.5	14 ± 0.6	15 ± 0.6	17 ± 0.7
Pseudomonas aeruginosa	11 ± 0.4	12 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Shigella spp.	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	14 ± 0.6	14 ± 0.6	15 ± 0.6	16 ± 0.7
Bacterial species	*Allium sativum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	15 ± 0.6	15 ± 0.6	16 ± 0.7
Enterococcus faecalis	Nil	Nil	Nil	Nil
Staphyloccus aureus	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Streptococcus pyogenes	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Escherichia coli	14 ± 0.6	14 ± 0.6	15 ± 0.6	16 ± 0.7
Klebsiella pneumonia	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Shigella spp.	14 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Vibrio cholerae	13 ± 0.5	15 ± 0.6	15 ± 0.6	16 ± 0.7
Bacterial species	*Zingiber officinale*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Staphyloccus aureus	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Streptococcus pyogenes	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Escherichia coli	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Klebsiella pneumonia	16 ± 0.7	17 ± 0.7	18 ± 0.8	19 ± 0.8
Pseudomonas aeruginosa	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Salmonella typhi	14 ± 0.6	15 ± 0.6	16 ± 0.7	18 ± 0.8
Shigella spp.	18 ± 0.8	19 ± 0.8	19 ± 0.8	19 ± 0.8
Vibrio cholerae	22 ± 0.9	22 ± 0.9	23 ± 1.0	24 ± 1.0
Bacterial species	*Curcuma longa*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Enterococcus faecalis	20 ± 0.9	20 ± 0.9	20 ± 0.9	21 ± 0.9
Staphyloccus aureus	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Streptococcus pyogenes	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Escherichia coli	26 ± 1.1	30 ± 1.3	31 ± 1.4	32 ± 1.4
Klebsiella pneumonia	11 ± 0.4	12 ± 0.5	12 ± 0.5	13 ± 0.5
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	14 ± 0.6	14 ± 0.6
Salmonella typhi	14 ± 0.6	14 ± 0.6	14 ± 0.6	18 ± 0.8
Shigella spp.	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Vibrio cholerae	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6

Table 10: Result of Chloroform extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Coriandrum sativum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	Nil	Nil	Nil	Nil
Staphyloccus aureus	12 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Streptococcus pyogenes	13 ± 0.5	15 ± 0.6	15 ± 0.6	15 ± 0.6
Escherichia coli	13 ± 0.5	15 ± 0.6	15 ± 0.6	15 ± 0.6
Klebsiella pneumonia	Nil	Nil	13 ± 0.5	14 ± 0.6
Pseudomonas aeruginosa	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Salmonella typhi	13 ± 0.5	15 ± 0.6	16 ± 0.7	17 ± 0.7
Shigella spp.	13 ± 0.5	14 ± 0.6	15 ± 0.6	16 ± 0.7
Vibrio cholerae	Nil	Nil	Nil	Nil
Bacterial species	*Trachyspermum ammi*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Enterococcus faecalis	15 ± 0.6	15 ± 0.6	16 ± 0.7	24 ± 1.0
Staphyloccus aureus	15 ± 0.6	17 ± 0.7	17 ± 0.7	18 ± 0.8
Streptococcus pyogenes	19 ± 0.8	20 ± 0.9	22 ± 0.9	22 ± 0.9
Escherichia coli	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	19 ± 0.8	19 ± 0.8
Pseudomonas aeruginosa	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Salmonella typhi	12 ± 0.5	12 ± 0.5	14 ± 0.6	15 ± 0.6
Shigella spp.	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Vibrio cholerae	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Bacterial species	*Cuminum cyminum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	12 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Staphyloccus aureus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Streptococcus pyogenes	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Escherichia coli	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	14 ± 0.6	18 ± 0.8	18 ± 0.8	18 ± 0.8
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	12 ± 0.5	12 ± 0.5
Salmonella typhi	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Shigella spp.	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Vibrio cholerae	25 ± 1.1	28 ± 1.1	29 ± 1.1	32 ± 1.1
Bacterial species	*Foeniculum vulgare*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	17 ± 0.7	18 ± 0.8	18 ± 0.8	18 ± 0.8
Staphyloccus aureus	11 ± 0.4	11 ± 0.4	11 ± 0.4	11 ± 0.4
Streptococcus pyogenes	19 ± 0.8	19 ± 0.8	19 ± 0.8	20 ± 0.9
Escherichia coli	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Klebsiella pneumonia	13 ± 0.5	13 ± 0.5	15 ± 0.6	16 ± 0.7
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Salmonella typhi	Nil	12 ± 0.5	14 ± 0.5	15 ± 0.6
Shigella spp.	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Vibrio cholerae	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8

Table 11. Result of Chloroform extract of spices against bacterial species

Bacterial species	Zone of inhibition in mm			
	Myristica fragrans			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Staphyloccus aureus	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Streptococcus pyogenes	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Escherichia coli	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Klebsiella pneumonia	17 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Pseudomonas aeruginosa	10 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Salmonella typhi	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Shigella spp.	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Vibrio cholerae	16 ± 0.7	16 ± 0.7	18 ± 0.8	19 ± 0.8
Bacterial species	*Trigonella foenum-graecum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	16 ± 0.7	16 ± 0.7	16 ± 0.7	18 ± 0.8
Staphyloccus aureus	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Streptococcus pyogenes	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Escherichia coli	10 ± 0.4	11 ± 0.4	12 ± 0.5	13 ± 0.5
Klebsiella pneumonia	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Pseudomonas aeruginosa	11 ± 0.4	12 ± 0.5	12 ± 0.5	14 ± 0.6
Salmonella typhi	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Shigella spp.	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Vibrio cholerae	15 ± 0.6	16 ± 0.7	16 ± 0.7	17 ± 0.7
Bacterial species	*Piper nigrum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	11 ± 0.4	11 ± 0.4	11 ± 0.4	13 ± 0.5
Staphyloccus aureus	12 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Streptococcus pyogenes	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Escherichia coli	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Klebsiella pneumonia	15 ± 0.6	15 ± 0.6	16 ± 0.7	18 ± 0.8
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Salmonella typhi	18 ± 0.8	18 ± 0.8	19 ± 0.8	20 ± 0.9
Shigella spp.	11 ± 0.4	12 ± 0.5	13 ± 0.5	13 ± 0.5
Vibrio cholerae	17 ± 0.7	18 ± 0.8	18 ± 0.8	20 ± 0.9
Bacterial species	*Syzygium aromaticum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	15 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Enterococcus faecalis	17 ± 0.7	18 ± 0.8	18 ± 0.8	19 ± 0.8
Staphyloccus aureus	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Streptococcus pyogenes	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Escherichia coli	18 ± 0.8	18 ± 0.8	18 ± 0.8	19 ± 0.8
Klebsiella pneumonia	Nil	11 ± 0.4	12 ± 0.5	13 ± 0.5
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Salmonella typhi	12 ± 0.5	13 ± 0.5	18 ± 0.8	19 ± 0.8
Shigella spp.	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6

Table 12: Result of Chloroform extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Cinnamomum zeylanicum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Enterococcus faecalis	14 ± 0.6	16 ± 0.7	16 ± 0.7	17 ± 0.7
Staphyloccus aureus	Nil	Nil	Nil	Nil
Streptococcus pyogenes	14 ± 0.6	14 ± 0.6	15 ± 0.6	16 ± 0.7
Escherichia coli	28 ± 1.2	30 ± 1.3	31 ± 1.4	35 ± 1.5
Klebsiella pneumonia	12 ± 0.5	12 ± 0.5	13 ± 0.5	15 ± 0.6
Pseudomonas aeruginosa	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Salmonella typhi	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Shigella spp.	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	11 ± 0.4	11 ± 0.4	11 ± 0.4	13 ± 0.5
Bacterial species	*Allium sativum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	12 ± 0.5	14 ± 0.6	16 ± 0.7	17 ± 0.7
Staphyloccus aureus	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Streptococcus pyogenes	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Escherichia coli	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	14 ± 0.6	15 ± 0.6	17 ± 0.7	17 ± 0.7
Salmonella typhi	18 ± 0.8	19 ± 0.8	19 ± 0.8	19 ± 0.8
Shigella spp.	17 ± 0.7	18 ± 0.8	18 ± 0.8	19 ± 0.8
Vibrio cholerae	14 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Bacterial species	*Zingiber officinale*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	14 ± 0.6	16 ± 0.7	16 ± 0.7	18 ± 0.8
Enterococcus faecalis	15 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Staphyloccus aureus	Nil	13 ± 0.5	13 ± 0.5	14 ± 0.6
Streptococcus pyogenes	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Escherichia coli	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	15 ± 0.6	17 ± 0.7	17 ± 0.7	18 ± 0.8
Salmonella typhi	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Shigella spp.	17 ± 0.7	17 ± 0.7	18 ± 0.8	19 ± 0.8
Vibrio cholerae	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Bacterial species	*Curcuma longa*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Enterococcus faecalis	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Staphyloccus aureus	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Streptococcus pyogenes	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Escherichia coli	25 ± 1.1	25 ± 1.1	27 ± 1.2	29 ± 1.3
Klebsiella pneumonia	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Salmonella typhi	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Shigella spp.	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Vibrio cholerae	12 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6

Table 13: Result of methanol extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Coriandrum sativum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	Nil	Nil	Nil	Nil
Staphyloccus aureus	8 ±0 .3	8 ± 0.3	10 ± 0.4	11 ± 0.4
Streptococcus pyogenes	11 ± 0.4	12 ± 0.5	12 ± 0.5	13 ± 0.5
Escherichia coli	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	Nil	11 ± 0.4	12 ± 0.5	13 ± 0.5
Pseudomonas aeruginosa	13 ± 0.5	16 ± 0.7	16 ± 0.7	16 ± 0.7
Salmonella typhi	15 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Shigella spp.	10 ± 0.4	14 ± 0.6	17 ± 0.7	17 ± 0.7
Vibrio cholerae	13 ± 0.5	14 ± 0.6	15 ± 0.6	17 ± 0.7
Bacterial species	*Trachyspermum ammi*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	11 ± 0.4	12 ± 0.5	13 ± 0.5	14 ± 0.6
Staphyloccus aureus	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Streptococcus pyogenes	16 ± 0.7	17 ± 0.7	18 ± 0.8	19 ± 0.8
Escherichia coli	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	13 ± 0.5	13 ± 0.5	14 ± 0.6	16 ± 0.7
Pseudomonas aeruginosa	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Salmonella typhi	17 ± 0.7	17 ± 0.7	17 ± 0.7	18 ± 0.8
Shigella spp.	18 ± 0.8	18 ± 0.8	18 ± 0.8	19 ± 0.8
Vibrio cholerae	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Bacterial species	*Cuminum cyminum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	13 ± 0.5	14 ± 0.6	16 ± 0.7
Enterococcus faecalis	15 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Staphyloccus aureus	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Streptococcus pyogenes	19 ± 0.8	19 ± 0.8	19 ± 0.8	19 ± 0.8
Escherichia coli	15 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Salmonella typhi	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Shigella spp.	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Vibrio cholerae	25 ± 1.1	26 ± 1.1	26 ± 1.1	26 ± 1.1
Bacterial species	*Foeniculum vulgare*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	15 ± 0.6	18 ± 0.8
Enterococcus faecalis	15 ± 0.6	16 ± 0.7	18 ± 0.8	18 ± 0.8
Staphyloccus aureus	10 ± 0.4	10 ± 0.4	10 ± 0.4	10 ± 0.4
Streptococcus pyogenes	17 ± 0.7	21 ± 0.9	21 ± 0.9	22 ± 0.9
Escherichia coli	12 ± 0.5	15 ± 0.6	16 ± 0.7	17 ± 0.7
Klebsiella pneumonia	18 ± 0.8	18 ± 0.8	18 ± 0.8	18 ± 0.8
Pseudomonas aeruginosa	14 ± 0.6	14 ± 0.6	16 ± 0.7	18 ± 0.8
Salmonella typhi	18 ± 0.8	19 ± 0.8	20 ± 0.9	20 ± 0.9
Shigella spp.	Nil	14 ± 0.6	15 ± 0.6	16 ± 0.7
Vibrio cholerae	18 ± 0.8	18 ± 0.8	19 ± 0.8	19 ± 0.8

Table 14: Result of Methanol extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Myristica fragrans			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	10 ± 0.4	11 ± 0.4	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	16 ± 0.7	16 ± 0.7	16 ± 0.7	16 ± 0.7
Staphyloccus aureus	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Streptococcus pyogenes	15 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Escherichia coli	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Klebsiella pneumonia	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Shigella spp.	11 ± 0.4	12 ± 0.5	13 ± 0.5	14 ± 0.6
Vibrio cholerae	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Bacterial species	*Trigonella foenum-graecum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Staphyloccus aureus	12 ± 0.5	12 ± 0.5	12 ± 0.5	13 ± 0.5
Streptococcus pyogenes	11 ± 0.4	11 ± 0.4	12 ± 0.5	13 ± 0.5
Escherichia coli	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Salmonella typhi	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Shigella spp.	13 ± 0.5	13 ± 0.5	13 ± 0.5	15 ± 0.6
Vibrio cholerae	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Bacterial species	*Piper nigrum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Staphyloccus aureus	12 ± 0.5	14 ± 0.6	15 ± 0.6	16 ± 0.7
Streptococcus pyogenes	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Escherichia coli	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Klebsiella pneumonia	15 ± 0.6	16 ± 0.7	16 ± 0.7	18 ± 0.8
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Shigella spp.	10 ± 0.4	10 ± 0.4	11 ± 0.4	12 ± 0.5
Vibrio cholerae	15 ± 0.6	17 ± 0.7	18 ± 0.8	19 ± 0.8
Bacterial species	*Syzygium aromaticum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	16 ± 0.7
Enterococcus faecalis	19 ± 0.8	20 ± 0.9	20 ± 0.9	20 ± 0.9
Staphyloccus aureus	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Streptococcus pyogenes	13 ± 0.5	15 ± 0.6	15 ± 0.6	15 ± 0.6
Escherichia coli	18 ± 0.8	18 ± 0.8	18 ± 0.8	19 ± 0.8
Klebsiella pneumonia	13 ± 0.5	14 ± 0.6	15 ± 0.6	16 ± 0.7
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	14 ± 0.6	13 ± 0.5
Salmonella typhi	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Shigella spp.	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Vibrio cholerae	13 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6

Table 15: Result of Methanol extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Cinnamomum zeylanicum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	15 ± 0.6	18 ± 0.8	19 ± 0.8	19 ± 0.8
Staphyloccus aureus	Nil	Nil	Nil	Nil
Streptococcus pyogenes	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Escherichia coli	17 ± 0.7	18 ± 0.8	18 ± 0.8	19 ± 0.8
Klebsiella pneumonia	19 ± 0.8	19 ± 0.8	19 ± 0.8	20 ± 0.9
Pseudomonas aeruginosa	11 ± 0.4	12 ± 0.5	12 ± 0.5	14 ± 0.6
Salmonella typhi	12 ± 0.5	14 ± 0.6	13 ± 0.5	15 ± 0.6
Shigella spp.	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Vibrio cholerae	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Bacterial species	*Allium sativum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	10±0.4	11±0.4	12±0.5	12±0.5
Enterococcus faecalis	Nil	Nil	Nil	Nil
Staphyloccus aureus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Streptococcus pyogenes	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ±0.6
Escherichia coli	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Klebsiella pneumonia	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Salmonella typhi	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Shigella spp.	12 ± 0.5	15 ± 0.6	16 ± 0.7	16 ± 0.7
Vibrio cholerae	Nil	Nil	12 ± 0.5	12 ± 0.5
Bacterial species	*Zingiber officinale*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	13 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Staphyloccus aureus	13 ± 0.5	16 ± 0.7	16 ± 0.7	17 ± 0.7
Streptococcus pyogenes	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Escherichia coli	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Klebsiella pneumonia	17 ± 0.7	18 ± 0.8	18 ± 0.8	19 ± 0.8
Pseudomonas aeruginosa	17 ± 0.7	18 ± 0.8	18 ± 0.8	19 ± 0.8
Salmonella typhi	18 ± 0.8	18 ± 0.8	19 ± 0.8	19 ± 0.8
Shigella spp.	18 ± 0.8	18 ± 0.8	19 ± 0.8	19 ± 0.8
Vibrio cholerae	25 ± 1.1	25 ± 1.1	26 ± 1.1	27 ± 1.2
Bacterial species	*Curcuma longa*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Enterococcus faecalis	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Staphyloccus aureus	13 ± 0.5	14 ± 0.6	15 ± 0.6	15 ± 0.6
Streptococcus pyogenes	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Escherichia coli	28 ± 1.2	28 ± 1.2	29 ± 1.3	29 ± 1.3
Klebsiella pneumonia	14 ± 0.6	15 ± 0.6	16 ± 0.7	16 ± 0.7
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	15 ± 0.6	16 ± 0.7
Salmonella typhi	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Shigella spp.	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Vibrio cholerae	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6

Table 16: Result of aqueous extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Coriandrum sativum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Enterococcus faecalis	Nil	Nil	Nil	Nil
Staphyloccus aureus	11 ± 0.5	11 ± 0.5	12 ± 0.5	12 ± 0.5
Streptococcus pyogenes	8 ± 0.3	9 ± 0.4	9 ± 0.4	10 ± 0.4
Escherichia coli	14 ± 0.6	14 ± 0.6	16 ± 0.7	16 ± 0.7
Klebsiella pneumonia	Nil	Nil	8 ± 0.3	8 ± 0.3
Pseudomonas aeruginosa	Nil	Nil	10 ± 0.4	11 ± 0.4
Salmonella typhi	13 ± 0.5	15 ± 0.6	15 ± 0.6	16 ± 0.7
Shigella spp.	14 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Vibrio cholerae	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Bacterial species	*Trachyspermum ammi*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Enterococcus faecalis	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Staphyloccus aureus	15 ± 0.6	16 ± 0.7	16 ± 0.7	16 ± 0.7
Streptococcus pyogenes	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Escherichia coli	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Klebsiella pneumonia	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Pseudomonas aeruginosa	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Salmonella typhi	13 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Shigella spp.	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Vibrio cholerae	15 ± 0.6	16 ± 0.7	16 ± 0.7	17 ± 0.7
Bacterial species	*Cuminum cyminum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	16 ± 0.7	18 ± 0.8	19 ± 0.8	20 ± 0.9
Staphyloccus aureus	Nil	Nil	12 ± 0.5	13 ± 0.5
Streptococcus pyogenes	20 ± 0.9	20 ± 0.9	21 ± 0.9	22 ± 0.9
Escherichia coli	15 ± 0.6	15 ± 0.6	15 ± 0.6	17 ± 0.7
Klebsiella pneumonia	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Pseudomonas aeruginosa	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Salmonella typhi	16 ± 0.7	17 ± 0.7	17 ± 0.7	17 ± 0.7
Shigella spp.	14 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Vibrio cholerae	15 ± 0.6	17 ± 0.7	17 ± 0.7	18 ± 0.8
Bacterial species	*Foeniculum vulgare*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	12 ± 0.5	14 ± 0.6	14 ± 0.6
Enterococcus faecalis	17 ± 0.7	17 ± 0.7	17 ± 0.7	18 ± 0.8
Staphyloccus aureus	Nil	9 ± 0.4	10 ± 0.4	10 ± 0.4
Streptococcus pyogenes	19 ± 0.8	20 ± 0.9	20 ± 0.9	21 ± 0.9
Escherichia coli	14 ± 0.6	16 ± 0.7	16 ± 0.7	17 ± 0.7
Klebsiella pneumonia	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Pseudomonas aeruginosa	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Salmonella typhi	14 ± 0.6	14 ± 0.6	16 ± 0.7	17 ± 0.7
Shigella spp.	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	17 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8

Table 17: Result of aqueous extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Myristica fragrans			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Enterococcus faecalis	16 ± 0.7	17 ± 0.7	18 ± 0.8	19 ± 0.8
Staphyloccus aureus	10 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Streptococcus pyogenes	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Escherichia coli	16 ± 0.7	17 ± 0.7	18 ± 0.8	18 ± 0.8
Klebsiella pneumonia	12 ± 0.5	12 ± 0.5	12 ± 0.5	13 ± 0.5
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	12 ± 0.5	12 ± 0.5
Salmonella typhi	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Shigella spp.	14 ± 0.6	14 ± 0.6	14 ± 0.6	15 ± 0.6
Vibrio cholerae	16 ± 0.7	16 ± 0.7	16 ± 0.7	17 ± 0.7
Bacterial species	*Trigonella foenum-graecum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Enterococcus faecalis	15 ± 0.6	16 ± 0.7	17 ± 0.7	17 ± 0.7
Staphyloccus aureus	10 ± 0.4	11 ± 0.4	11 ± 0.4	11 ± 0.4
Streptococcus pyogenes	9 ± 0.4	10 ± 0.4	11 ± 0.4	11 ± 0.4
Escherichia coli	12 ± 0.5	12 ± 0.5	12 ± 0.5	13 ± 0.5
Klebsiella pneumonia	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6
Salmonella typhi	10 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Shigella spp.	12 ± 0.5	13 ± 0.5	13 ± 0.5	13 ± 0.5
Vibrio cholerae	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Bacterial species	*Piper nigrum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	11 ± 0.4	11 ± 0.4	11 ± 0.4	14 ± 0.6
Enterococcus faecalis	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Staphyloccus aureus	11 ± 0.4	11 ± 0.4	11 ± 0.4	12 ± 0.5
Streptococcus pyogenes	11 ± 0.4	12 ± 0.5	12 ± 0.5	12 ± 0.5
Escherichia coli	14 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Klebsiella pneumonia	15 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Pseudomonas aeruginosa	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Salmonella typhi	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Shigella spp.	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Vibrio cholera	14 ± 0.6	14 ± 0.6	14 ± 0.6	14 ± 0.6
Bacterial species	*Syzygium aromaticum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	17 ± 0.7	19 ± 0.8	20 ± 0.9	21 ± 0.9
Enterococcus faecalis	18 ± 0.8	19 ± 0.8	20 ± 0.9	20 ± 0.9
Staphyloccus aureus	16 ± 0.7	19 ± 0.8	20 ± 0.9	20 ± 0.9
Streptococcus pyogenes	15 ± 0.6	17 ± 0.7	18 ± 0.8	19 ± 0.8
Escherichia coli	16 ± 0.7	18 ± 0.8	18 ± 0.8	18 ± 0.8
Klebsiella pneumonia	14 ± 0.6	18 ± 0.8	18 ± 0.8	19 ± 0.8
Pseudomonas aeruginosa	15 ± 0.6	15 ± 0.6	15 ± 0.6	16 ± 0.7
Salmonella typhi	16 ± 0.7	16 ± 0.7	19 ± 0.8	20 ± 0.9
Shigella spp.	13 ± 0.5	14 ± 0.6	16 ± 0.7	19 ± 0.8
Vibrio cholera	18 ± 0.8	18 ± 0.8	19 ± 0.8	19 ± 0.8

Table 18: Result of aqueous extract of spices against bacterial species.

Bacterial species	Zone of inhibition in mm			
	Cinnamomum zeylanicum			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	14 ± 0.6	15 ± 0.6	16 ± 0.7	17±0.7
Enterococcus faecalis	15 ± 0.6	15 ± 0.6	16 ± 0.7	17±0.7
Staphyloccus aureus	Nil	Nil	Nil	Nil
Streptococcus pyogenes	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Escherichia coli	31 ± 1.3	32 ± 1.4	34 ± 1.5	35 ± 1.5
Klebsiella pneumonia	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Salmonella typhi	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Shigella spp.	12 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Vibrio cholerae	12 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Bacterial species	*Allium sativum*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	Nil	Nil	12 ± 0.5	12 ± 0.5
Enterococcus faecalis	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Staphyloccus aureus	13 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Streptococcus pyogenes	13 ± 0.5	13 ± 0.5	14 ± 0.6	15 ± 0.6
Escherichia coli	12 ± 0.5	13 ± 0.5	14 ± 0.6	16 ± 0.7
Klebsiella pneumonia	17 ± 0.7	17 ± 0.7	17 ± 0.7	19 ± 0.8
Pseudomonas aeruginosa	15 ± 0.6	15 ± 0.6	15 ± 0.6	15 ± 0.6
Salmonella typhi	17 ± 0.7	18 ± 0.8	19 ± 0.8	19 ± 0.8
Shigella spp.	17 ± 0.7	18 ± 0.8	19 ± 0.8	19 ± 0.8
Vibrio cholerae	Nil	15 ± 0.6	16 ± 0.7	16 ± 0.7
Bacterial species	*Zingiber officinale*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	Nil	12 ± 0.5	12 ± 0.5	14 ± 0.6
Enterococcus faecalis	13 ± 0.5	14 ± 0.6	14 ± 0.6	14 ± 0.6
Staphyloccus aureus	14 ± 0.6	14 ± 0.6	15 ± 0.6	15 ± 0.6
Streptococcus pyogenes	16 ± 0.7	16 ± 0.7	17 ± 0.7	17 ± 0.7
Escherichia coli	15 ± 0.6	15 ± 0.6	17 ± 0.7	17 ± 0.7
Klebsiella pneumonia	14 ± 0.6	15 ± 0.6	17 ± 0.7	18 ± 0.8
Pseudomonas aeruginosa	17 ± 0.7	18 ± 0.8	18 ± 0.8	18 ± 0.8
Salmonella typhi	16 ± 0.7	16 ± 0.7	17 ± 0.7	18 ± 0.8
Shigella spp.	16 ± 0.7	16 ± 0.7	17 ± 0.7	17± 0.7
Vibrio cholerae	26 ± 1.1	26 ± 1.1	28 ± 1.2	28 ± 1.2
Bacterial species	*Curcuma longa*			
	50 µL	100 µL	150 µL	200 µL
Bacillus cereus	13 ± 0.5	14 ± 0.6	14 ± 0.6	15 ± 0.6
Enterococcus faecalis	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Staphyloccus aureus	12 ± 0.5	13 ± 0.5	13 ± 0.5	14 ± 0.6
Streptococcus pyogenes	10 ± 0.4	11 ± 0.4	12 ± 0.5	10 ± 0.4
Escherichia coli	34 ± 1.5	34 ± 1.5	34 ± 1.5	35 ± 1.5
Klebsiella pneumonia	14 ± 0.6	15 ± 0.6	16 ± 0.7	17 ± 0.7
Pseudomonas aeruginosa	12 ± 0.5	12 ± 0.5	12 ± 0.5	13 ± 0.5
Salmonella typhi	13 ± 0.5	13 ± 0.5	14 ± 0.6	14 ± 0.6
Shigella spp.	12 ± 0.5	12 ± 0.5	13 ± 0.5	13 ± 0.5
Vibrio cholerae	12 ± 0.5	12 ± 0.5	13 ± 0.5	14 ± 0.6

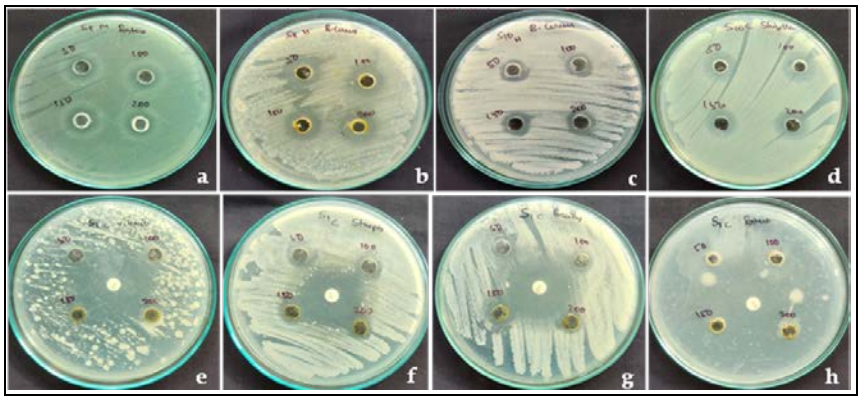

Figure 2.a: *Enterococcus faecalis* in *S. aromaticum* extract, b. *Bacillus cereus* in *S. aromaticum* extract, c. *Bacillus cereus* in *A. sativum* extract, d. *Shigella* sp. in *A. sativum* extract, e. *Vibrio cholerae* in Erythromycin (E-15µg), f-h: Bacterial species showed no activity in Erythromycin (E-15µg) - *Streptococcus py*ogenes, g. *Bacillus cereus*, h. *Enterococcus faecalis.*

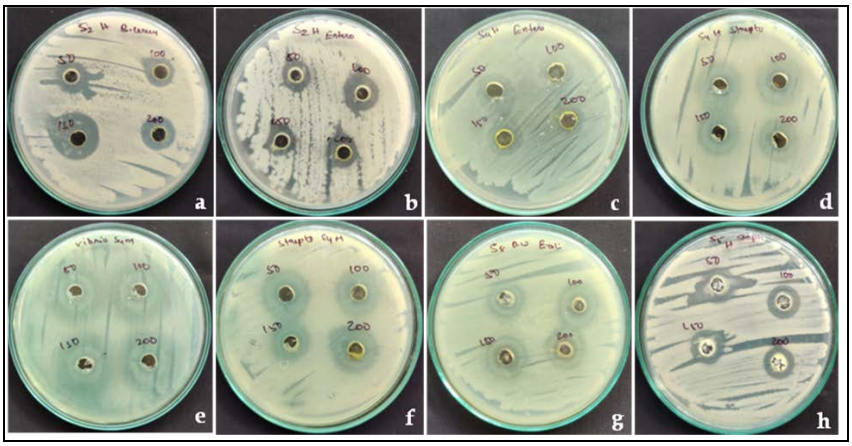

Figure 3.a: *Bacillus cereus* in T. *ammi* extract, b. *Enterococcus faecalis* in *T. ammi extract*, c. *Enterococcus faecalis* in *F. vulgare* extract, d. *Streptococcus pyogenes* in *F. vulgare* extract, e. *Vibrio cholerae* in *F. vulgare* extract, f. Sg. *Escherichia coli* in *M. fragrans* extract, h. *Staphylococcus aureus* in *M. fragrans* extract

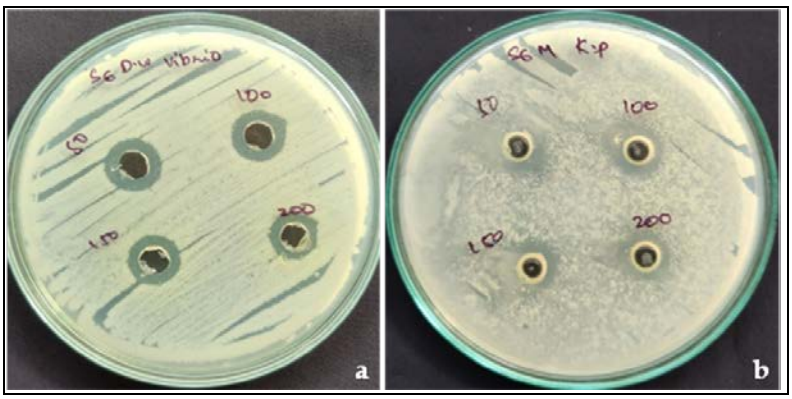

Figure 4: *Vibrio cholerae* in *T. foenum-graceum* extract,
b. *Klebsiella pneumoniae* in *T. foenum-gracum* extract.

Activity of hexane extract

The results of antibacterial activity of hexane extract of all twelve spices showed a varying degree of inhibition on each tested pathogenic bacterium and the zone of inhibition ranged from 10 ± 0.4 to 32 ± 1.4 mm. The hexane extract of *F. vulgare* showed the minimum zone of inhibition at 50 µl/mL concentration against *S. aureus*, while that of *C. zeylanicum* and *C. longa* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli*. In 50 µl/mL concentration of hexane extract, the zone of inhibition ranged from 10 ± 0.4 to 26 ± 1.1 mm against all ten pathogenic bacteria. The hexane extract of *C. cyminum* and *F. vulgare* showed the minimum zone of inhibition against *S. aureus* while that of *C. cyminum* showed the maximum zone of inhibition against *V. cholerae* and also that of *C. longa* against *E. coli.* The hexane extract of *T. foenum-graecum* and *C. zeylanicum* did not show any zone of inhibition against *S. aureus*. Further, the hexane extract of *A. sativum* also did not show any zone of inhibition against *E. faecalis*.

In 100 µl/mL concentration of hexane extract, the zone of inhibition ranged from 10 ± 0.4 to 31 ± 1.3 mm against all ten pathogenic bacteria. The minimum zone of inhibition against *S. aureus* was observed with the hexane extract of *F. vulgare* while the maximum zone of inhibition against *E. coli* was observed with the hexane extract of *C. zeylanicum*. No zone of inhibition against *S. aureus* was observed with the hexane extract of *C. zeylanicum* and against *E. faecalis* with the hexane extract of *A. sativum.*

In 150 µl/mL concentration of hexane extract, the zone of inhibition ranged from 11 ± 0.4 to 31 ± 1.3 mm against all ten pathogenic bacteria. The minimum zone of inhibition against *S. aureus* was observed with the hexane extract of *C. cyminum* and *F. vulgare* while the maximum zone of inhibition against *E. coli* was observed with the hexane extract of *C. zeylanicum* and *C. longa*. No zone of inhibition against *S. aureus* was observed with the hexane extract of *C. zeylanicum* and against *E. faecalis* with the hexane extract of *A. sativum.*

In 200 µl/mL concentration of hexane extract, the zone of inhibition ranged from 12 ± 0.5 to 32 ± 1.4 mm against all ten pathogenic bacteria. The minimum zone of inhibition against *E. faecalis* was observed with the hexane extract of *C. sativum,* against *S. aureus* with the extract of *C. cyminum, F. vulgare* and *S. aromaticum*, and against *B. cereus* with the extract of *C. cyminum*. The maximum zone of inhibition against *E. coli* was observed with the hexane extract of *C. zeylanicum* and *C. longa*. A moderate zone of inhibition against *V. cholerae* was observed with the extract of *C. cyminum* and *Z. officinale* and against *K. pneumoniae* with the extract of *T. ammi* and *S. aromaticum*. No zone of inhibition against *E. faecalis* was observed with the extract of *C. zeylanicum* and *A. sativum*.

Activity of chloroform extract

The results of antibacterial activity of chloroform extract of all twelve spices showed varying degree of inhibition on each tested pathogenic bacterium and the zone of inhibition ranged from 10 ± 0.4 to 35 ± 1.5 mm. The chloroform extract of *T. foenum-graceum* showed the minimum zone of inhibition at 50 µl/mL concentration against *E. coli* while that of *C. zeylanicum* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli.* The maximum zone of inhibition was also observed against *V. cholerae* with 200 µl/mL concentration of *C. cyminum*.

In 50 µl/mL concentration of chloroform extract, the zone of inhibition ranged from 10 ± 0.4 to 28 ± 1.2 mm against all ten pathogenic bacteria. The chloroform extract of *M. fragrans* showed the minimum zone of inhibition against *P. aeruginosa* while that of *C. zeylanicum* showed the maximum zone of inhibition against *E. coli*. No zone of inhibition against *E. faecalis, K. pneumonia,* and *V. cholerae* with the extract of *C. sativum,* against *S.*

aureus with the extract of *C. zeylanic*um and *Z. officinale* and against *S. typhi* with the extract of *F. vulgare* and against *K. pneumoniae* with the extract of *S. aromaticum*.

In 100 µl/mL concentration of chloroform extract, the zone of inhibition ranged from 11 ± 0.4 to 30 ± 1.3 mm against all ten pathogenic bacteria. The minimum zone of inhibition was observed against *S. aureus* with the extract of *F. vulgare*, against *P. aeruginosa* with the extract of *M. fragrans*, against *E. coli* with the extract of *T. foenum-graecum*, against *E. faecalis* with the extract of *P. nigrum*, against *K. pneumoniae* with the extract of *S. aromaticum* and against *V. cholerae* with the extract of *C. zeylanicum*. The maximum zone of inhibition against *E. coli* with the extract of *C. zeylanicum*. No zone of inhibition against *E. faecalis*, *K. pneumonia*, and *V. cholerae* was observed with the extract of *C. sativum* and against *S. aureus* with the extract of *C. zeylanicum*.

In 150 µl/mL concentration of chloroform extract, the zone of inhibition ranged from 11 ± 0.4 to 31 ± 1.3 mm against all ten pathogenic bacteria. The maximum zone of inhibition against *E. coli* was observed with the extract of *C. zeylanicum*. The minimum zone of inhibition was observed against *S. aureus* with the extract of *F. vulgare*, against *P. aeruginosa* with the extract of *M. fragrans*, against *E. faecalis* with the extract of *P. nigrum* and against *V. cholerae* with the extract of *C. zeylanicum*. No zone of inhibition was observed against *E. faecalis*, and *V. cholerae* with the extract of *C. sativum* and against *S. aereus* with the extract of *C. zeylanicum.*

In 200 µl/mL concentration of chloroform extract, the zone of inhibition ranged from 11 ± 0.4 to 35 ± 1.5 mm against all ten pathogenic bacteria. The maximum zone of inhibition was observed against *E. coli* with the extract of *C. zeylanicum.* A moderate zone of inhibition was observed against *V. cholerae* with the extract of *C. cyminum*, against *E. coli* with the extract of *C. longa* and against *S. pyogenes* with the extract of *T. ammi*. The minimum zone of inhibition was observed against *S. aureus* with the extract of *F. vulgare.* No zone of inhibition was observed against *E. faecalis* and *V. cholerae* with the extract of *C. sativum* and against *S. aureus* with the extract of *C. zeylanicum*.

Activity of methanol extract

The results of antibacterial activity of methanol extract of all twelve spices showed a varying degree of inhibition on each tested pathogenic bacterium and the zone of inhibition ranged from 8 ± 0.3 to 29 ± 1.3 mm. The chloroform extract of *C. sativum* showed the minimum zone of inhibition at 50 µl/mL concentration against *S. aureus*, while that of *C. longa* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli*.

In the 50 µl/mL concentration of methanol extract, the zone of inhibition ranged from 10 ± 0.4 to 28 ± 1.2 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. aureus* with the extract of *C. sativum* while the maximum zone of inhibition was observed against *V. cholerae* with the extracts of *C. cyminum* and *Z. officinale*. No zone of inhibition was observed against *E. faecalis* and *K. pneumoniae* with the extract of *C. sativum*, against *Shigella* sp. with the extract of *F. vulgare* and against *S. aureus* with the extract of *C. zeylanicum* and against *E. faecalis* with the extract of *A. sativum*.

In 100 µl/mL concentration of methanol extract of all twelve spices, the zone of inhibition ranged from 8 ± 0.3 to 26 ± 1.1 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. aereus* with the extract of *C. sativum* while the maximum zone of inhibition was observed against *E. coli* with the extract of *C. longa*. No zone of inhibition was observed against *E. faecalis* with the extracts of *C. sativum* and *A. sativum* and against *S. aureus* with the extract of *C. zeylanicum.*

In 150 µl/mL concentration of methanol extract of all twelve spices, the zone of inhibition ranged from 10 ± 0.4 to 29 ± 1.3 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. aureus* with the extracts of *C. sativum* and *F. vulgare*, while the maximum zone of inhibition was observed against *E. coli* with the extract of *C. longa*. No zone of inhibition was observed against *E. faecalis* with the extracts of *C. sativum* and *A. sativum* and against *S. aureus* with the extract of *C. zeylanicum*.

In 200 µl/mL concentration of methanol extract of all twelve spices, the zone of inhibition ranged from 10 ± 0.4 to 29 ± 1.3 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. aureus* with the extract of *F. vulgare.* The maximum zone of inhibition was observed against *E. coli* with the extract of *C. longa.* A moderate zone of inhibition was observed against *V. cholerae* with the extracts of *C. cyminum* and *Z. officinale*, against *S. pyogenes* with the extract of *F. vulgare*, against *K. pneumoniae* with the extract of *C. zeylanicum*, against *E. faecalis* with the extract of *S. aromaticum* and against *S. typhi* with the extract of *C. cyminum.* No zone of inhibition was observed against *E. faecalis* with the extracts of *C. sativum* and *A. sativum* and against *S. aureus* with the extract of *C. zeyla*nicum.

Activity of aqueous extract

The results of antibacterial activity of aqueous extract of all twelve spices showed varying degree of inhibition on each tested pathogenic bacterium and the zone of inhibition ranged from 8 ± 0.3 to 35 ± 1.5 mm. The aqueous extract of *C. sativum* showed the minimum zone of inhibition at 50 µl/mL concentration against *S. pyogenes*, while that of *C. zeylanicum* and *C. longa* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli*.

In 50 µl/mL concentration of aqueous extract, the zone of inhibition ranged from 8 ± 0.3 to 34 ± 1.5 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. pyogenes* with the extract of *C. sativum*, while the maximum zone of inhibition was observed against *E. coli* with the extracts of *C. longa*. No zone of inhibition was observed against *E. faecalis, K. pneumonia*, and *P. aeruginosa* with the extract of *C. sativum*, against *S. aureus* with the extracts of *F. vulgare* and *C. zeylanicum*, against *B. cereus* and *V. cholerae* with the extract of *A. sativum* and against *B. cereus* with the extract of *Z. officinale*.

In 100 µl/mL concentration of aqueous extract, the zone of inhibition ranged from 9 ± 0.4 to 34 ± 1.5 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *S. pyogenes* with the extract of *C. sativum* and against *S. aureus* with the extract of *F. vulgare.* The maximum zone of inhibition was observed against *E. coli* with the extract of *C. longa*. No zone of inhibition was observed against E. *faecalis, K. pneumonia*, and *P. aeruginosa* with the extract of *C. sativum*, against *S. aureus* with the extracts of *C. cyminum* and *C. zeylanicum* and against *B. cereus* with the extract of *A. sativum*.

In 150 µl/mL concentration of aqueous extract, the zone of inhibition ranged from 9 ± 0.4 to 34 ± 1.5 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *K. pneumoniae* with the extract of *C. sativum,* while the maximum zone of inhibition was observed against *E. coli* with the extracts of *C. zeylanicum* and *C. longa*. No zone of inhibition was observed against *E. faecalis* with the extract of *C. sativum* and against *S. aureus* with the extract of *C. zeylanicum.*

In 200 µl/mL concentration of aqueous extract, the zone of inhibition ranged from 8 ± 0.3 to 35 ± 1.5 mm against all ten bacterial pathogens. The minimum zone of inhibition was observed against *K. pneumoniae* with the extract of *C. sativum.* The maximum zone of inhibition was observed against *E. coli* with the extract of *C. longa* and *C. zeylanicum.* The moderate zone of inhibition was observed against *V. cholerae* with the extract of *Z. officinale*,

against *S. pyogenes* with the extract of *C. cyminum* and *F. vulgare*, against *B. cereus, E. faecalis, S. aureus*, and *S. typhi* with the extract of *S. aromaticum*. No zone of inhibition was observed against *E. faecalis* with the extract of *C. sativum* and against *S. aureus* with the extract of *C. zeylanicum.*

The study indicated that the extracts of *C. zeylanicum* and *C. longa* showed highest antibacterial activity against *E. coli*. The activity of cinnamon is due to the presence of cinnamaldehyde, an aromatic aldehyde that inhibits amino acid decarboxylase activity and active against many pathogenic bacteria (Charu et al., 2008). The bacterial strains tested in the study belonged to different genera. *Bacillus subtilis* is a Gram-positive spore forming rods; *Staphylococcus aureus* is a Gram-positive cocci and *Escherichia coli* is a Gram-negative *Enterobacteria*. The findings of this study showed that *C. zeylanicum* bark extract is effective to inhibit both Gram-positive and Gram-negative bacteria and has broad spectrum inhibitory effect. Gram positive bacteria are more susceptible than Gram-negative bacteria to the action of *C. zeylanicum* bark extract, demonstrating an antibacterial effect that is comparable to that of the standard drug, Erythromycin.

There are several reports in the literature indicating the antibacterial and antifungal activity of the medicinal plants. Many studies reported the incapability of herbal antimicrobial agents to inhibit growth of Gram-negative bacteria (Varalakshmi et al., 2014) due to the presence of a complex cell wall structure, which decreases the penetration of bacterial cells by herbal extracts. But in the present study, *Cinnamomum zeylanicum* bark extract showed inhibition at a moderate level on the growth of *E. coli*, indicating that this bark extract has the penetrating ability for bacterial cells. The present study reports that hexane, methanol, and aqueous extracts of *C. longa* showed highest activity against *E. coli*. Different authors reported that *C. longa* has pharmacological uses against skin diseases, as well as anti-microbial, anti-diabetic, anti-oxidant, anti-protozoal, anti-microbial, anti-venom, anti-tumor, anti-inflammatory, hapatoprotective, anti-allergic, anti-ulcer, anti-dyspeptic, and anti-depressant qualities (Afaf et al., 2006; Rudrappa and Bais, 2008). Further, in this study, the chloroform extract of *C. cyminum* has the highest antibacterial activity against *V. cholerae.* The aqueous extract of this spice has also been reported to inhibit the growth of *E. coli, S. aureus, Salmonella*, and *B. cereus* (Chaudhry and Tariq, 2008).

Minimum inhibitory concentration (MIC)

The dilution method is mainly useful in determining minimum inhibitory concentration. It is the least concentration of antimicrobial agent that prevents microbial growth as well as the determination of minimum bactericidal concentration (MBC), which is the least concentration of antimicrobial agent required to kill microorganisms (Andrews, 2001). According to MIC results in this study, 0-14% concentration is the minimum inhibitory concentration to inhibit the growth of bacterial strains. Among all twelve spices, *Z. officinale, P. nigrum*, and *T. foenum-graecum* extracts inhibit the growth of *S. pyogenes, E. coli*, and *S. typhi* respectively.

CONCLUSIONS

The study pertains to the isolation of pathogenic bacteria from three commercially edible tuna fishes, *Euthynnus affinis, Katsuwonus pelamis*, and *Auxis thazard*. The isolated bacterial species included *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus*, and *Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Shigella* sp., and *Vibrio cholerae*. According to MPN Method, the highest coliform count in *E. affinis* was recorded during November 2018 and September 2019, in *K.*

pelamis during September 2019 and in *A. thazard* during March and April 2019. The lowest coliform count in *E. affinis* was recorded during March 2019, in *K. pelamis* during March 2018 and in *A. thazard* during November 2018 and September 2019. MPN values of these fish species indicated that *K. pelamis* has the highest total bacterial count and the high counts of faecal coliforms, indicating contamination of sea water/selling point with the pathogenic bacteria. TVC analysis indicated that *E. affinis* has the highest percentage followed by *K. pelamis* and *A. thazard*. TPC analysis showed that *E. affinis* has the highest mean value of bacteria count, while *A. thazard* has the lowest mean value of bacteria count. Monthly TPC values indicated that *E. affinis* has the highest count in July 2019, *K. pelamis* in February 2019 and *A. thazard* in October 2018. Further, *E. affinis* has the lowest count in November 2018, *K. pelamis* in April 2019 and *A. thazard* in April 2019.

In all the three studied fish species, the percentage of bacterial populations ranged from 7% to 11%. Among these bacteria, *S. aureus* is the major causative agent for food poisoning in humans as it is known to release entero-toxins which cause severe illness in the gastro-intestinal tract. *E. coli, E. faecalis*, *P. Aeruginosa*, and *B. cereus* showed moderate counts in different months while *K. pneumoniae, S. typhi, Shigella* spp., and *V. cholerae* showed low counts in all the three fish samples. The presence of bacterial pathogens is attributed to the contamination of the sea water, fish processing, improper handling, hygienic, and sanitary conditions of Visakhapatnam Harbor.

Phyto-chemical analysis of the selected parts of spices indicated that alkaloids and flavonoids are present in all twelve spices. Phenols, quinones, saponins, and steroids are absent in *Z. officinale*; in this species, rhizome paste solvent is positive for phenols while rhizome powder solvent is negative for phenols. *A. sativum* is negative for tannins, *S. aromaticum* for quinones, *C. zeylanicum* for carbohydrates, and *F. vulgare, T. foenum-graecum, C. Zeylanicum*, and *C. longa* are negative for proteins. *C. sativum* is negative for quinones and steroids, and *P. nigrum* for quinones, terpenoids, and steroids. *T. ammi* is negative for tannins, terpenoids, and steroids. *C. cyminum* is negative for tannins, cardiac glycosides, proteins, and saponins.

In vitro antibacterial activity of hexane extract of all concentrations of all spices collectively showed the zone of inhibition ranging from 10 ± 0.4 to 32 ± 1.4 mm; *F. vulgare* showed the minimum zone of inhibition at 50 µl/mL concentration against *S. aureus* and *C. zeylanicum* and *C. longa* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli.* The chloroform extract of all concentrations of all spices collectively showed the zone of inhibition ranging from 10 ± 0.4 to 35 ± 1.5 mm; *T. foenum-graceum* showed the minimum zone of inhibition at 50 µl/mL concentration against *E. coli* and *C. zeylanicum* showed the maximum zone of inhibition against *E. coli* while *C. cyminum* against *V. cholerae* at 200 µl/mL concentration. The methanol extract of all concentrations of all spices collectively showed the zone of inhibition ranging from 8 ± 0.3 to 29 ±1.3 mm; *C. sativum* showed the minimum zone of inhibition at 50 µl/mL concentration against *S. aureus* and *C. longa* showed the maximum inhibition at 200 µl/mL concentration against *E. coli.* The aqueous extract of all concentrations of all spices collectively showed the zone inhibition ranging from 8 ± 0.3 to 35 ± 1.5 mm; *C. sativum* showed the minimum inhibition at 50 µl/mL concentration against *S. pyogenes* and C. *zeylanicum* and *C. longa* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli.* All four solvent extracts of all spices showed the extent of inhibition varying from 8 ± 0.3 to 35 ± 1.5 mm. The maximum zone of inhibition (35 mm) was observed in 200 µl/mL concentration of each form of extract. The chloroform and aqueous extracts of *C. zeylanicum* showed the maximum zone of inhibition at 200 µl/mL concentration against *E. coli* while methanol and aqueous extracts of *C. sativum* showed the minimum zone of inhibition (8 mm) at 50 µl/mL concentration against *S. aureus* and *S. pyogenes*.

The study finds that the maximum antibacterial activity by *C. zeylanicum* and *C. longa* against *E. coli* is attributed to the presence of an aromatic cinnamaldehyde that inhibits amino acid decarboxylase activity and is active against many pathogenic bacteria. *C. zeylanicum* bark extract is effective to inhibit both Gram-positive and Gram-negative bacteria and has broad spectrum inhibitory effect. Gram-positive bacteria are more susceptible than Gram-negative bacteria to the action of *C. zeylanicum* bark extract demonstrating an antibacterial effect that is comparable to that of the standard drug Erythromycin. Further, the moderate level inhibition by *C. zeylanicum* bark extract on the growth of *E. coli* indicates that this bark extract has the penetrating ability for bacterial cells. The hexane, methanol, and aqueous extracts of *C. longa* are highly effective against *E. coli* while chloroform extract of *C. cyminum* is highly effective against *V. cholerae*. The spice extracts are very promising candidates for further reseach to use them to control pathogenic bacteria.

The study indicates that all three tuna fish species are contaminated microbiologically and the bacterial species isolated are pathogenic and can cause various human health problems upon consumption when the fishes are uncooked. It is suggested that sea water contamination is to be regulated and effective steps to monitor and maintain sanitary conditions at fish landing points/selling points. Continued consumption of these fishes in large enough numbers without proper processing and cooking could cause serious illnesses in humans.

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FISH FAUNA OF ILISU AREA ON THE TIGRIS RIVER, BEFORE IMPOUNDMENT OF THE ILISU DAM (TURKEY)

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ABSTRACT

The Ilisu Dam is a large hydroelectric power plant that started to collect water in 2019 on the Tigris River in Turkey. This study was done during the period 2010 to 2014 to determine fish fauna of the Tigris River and its tributaries related to the potential reservoir area of the Ilisu Dam before its foundation. 35 fish species belonging to 10 families have been identified in the main river and its tributaries to be covered by the dam reservoir. One of these species, *Glyptothorax steindachneri* was recorded for the first time from the Turkish part of the Tigris River. 22 species were found widely distributed. At least 16 species were found to be endemic to the Tigris and Euphrates basins. Three species were already recommended with some level of international protection as per IUCN Red List. *Cobitis kellei* and *Paraschistura chrysicristinae* species, whose type localities were reported as upper Tigris, were not recorded.

RÉSUMÉ: Ichtyofaune de la zone d'Ilisu sur le Tigris River, avant la mise en cale du barrage d'Ilisu (Turquie).

Le barrage d'Ilisu est une grande centrale hydroélectrique qui a commencé à recueillir de l'eau en 2019 sur la rivière Tigris en Turquie. Cette étude a été réalisée entre 2010 et 2014 pour déterminer la faune des poissons de la rivière Tigris et de ses affluents liés à la zone du réservoir potentiel du barrage Ilisu avant sa fondation. 35 espèces de poissons appartenant à 10 familles ont été identifiées dans la rivière principale et ses affluents pour être couverts par le réservoir du barrage. Une de ces espèces, *Glyptothorax steindachneri*, a été enregistrée pour la première fois dans la partie turque du Tigre. 22 espèces ont été trouvées largement réparties. Au moins 16 espèces ont été trouvées endémiques du bassin du Tigre et de l'Euphrate. Trois espèces ont déjà été recommandées avec un certain niveau de protection internationale, conformément à la Liste Rouge de l'UICN. Les espèces *Cobitis kellei* et *Paraschistura chrysicristinae*, dont les localités types ont été signalées comme le Tigre supérieur, n'ont pas été consignées dans cette étude.

REZUMAT: Fauna ihtiologică din zona Ilisu de pe râul Tigru, înainte de realizarea barajului Ilsu (Turcia).

Barajul Ilisu este o hidrocentrală de mari dimensiuni care a început să colecteze apă în 2019 pe râul Tigru din Turcia. Acest studiu a fost realizat în perioada dintre 2010 şi 2014 pentru a determina fauna ihtiologică din zona Ilisu a râului Tigru în amonte de barajul Ilisu înainte de construirea acestuia. 35 de specii de peşti aparţinând a 10 familii au fost identificate în râul principal şi afluenţii săi ce sunt acoperite de rezervorul barajului. Una dintre aceste specii, *Glyptothorax steindachneri* a fost înregistrată pentru prima dată pe partea turcească a râului Tigru. 22 de specii au fost găsite distribuite pe scară largă. Cel puţin 16 au fost găsite endemice în bazinele Tigru şi Eufrat. Trei specii au fost deja recomandate cu un anumit nivel de protecţie internaţională conform Listei Roşii a IUCN. Speciile *Cobitis kellei* şi *Paraschistura chrysicristinae*, ale căror locaţii tipice au fost raportate ca fiind Tigrul superior, nu au fost înregistrate în acest studiu.

INTRODUCTION

Dams built on rivers, water pollution and excessive use of water due to agricultural activities, are among the most important threats on fish biodiversity (Akama, 2017). While dams provide economic benefits due to flood protection, water supply, and renewable energy contributions, they also cause adverse effects as they cause hydrological change of freshwater ecosystems. In the impounded area of the dams, the main impact is the change from lotic to lentic water, which influences aquatic fauna, including fishes (Jackson and Marmulla, 2001; Agostinho et al., 2008). In addition, the river downstream differs completely from it's old structure in terms of water quality entering its reservoir, water flow, sediment load, and water temperature (Granzotti et al., 2018). As a result, completely new riverside vegetation is formed and natural fish species are highly affected by this new structure of the river. (Collier et al., 1996). Dams also threaten the future of the species by preventing the movement or migration of fish along the river to spawn and feed (Larinier, 2001; Barbarossa et al., 2020). In addition, they prevent the distribution and spread of fish species in the aquatic ecosystem and their tendency to new habitats. In order to evaluate the effects of changes in current and future freshwater ecosystems on fish biodiversity and to take necessary precautions, it is very important to determine the current fish species, to reveal their distribution and habitat structures, to identify and evaluate conservation needs.

Tigris River rises from Hazar Lake in the Tauras Mountains in southeastern Turkey. Fed by many streams coming from the Taurus Mountains the river flows along Turkey-Syria border before entering into Iraq. By joining with the Euphrates River near Al-Qurnah in Iraq, it takes the name Shatt Al-Arab and flows into the Persian Gulf (Al-Ansari et al., 2018). Within the borders of Turkey, Kralkiz, Dicle, and Ilisu dams and hydropower projects are in operation on the Tigris River. The largest of these, Ilisu, is located on the main river course, 65 km upstream from the border line between Iraq and Syria (Eberlein et al., 2010; Al-Madhhachi et al., 2020).

In the upper section of the Tigris River in Turkey, a few studies on fish fauna of the river have made contributions on the taxonomy and distribution of fish species (Kuru, 1975, 1979; Ünlü, 1999; Ünlü, 2006; Ünlü et al., 2011; Ünlü, 2014; Ünlü et al., 2017). Many new species have been identified in this section of the River (Bănărescu and Nalbant, 1964; Nalbant, 1998; Bogutskaya, 1995; Erk'akan et al., 1998; Turan et al., 2011, 2016; Freyhof and Özuluğ, 2017; Freyhof et al., 2017, 2018). Recently, 40 species of fish belonging to 10 families have been recorded in the upper basin of the Tigris River in Turkey (Kaya et al., 2016).

Despite the high fish diversity of the Tigris River, there have been significant changes in fish distribution and community structure due to dams. There are no fish passes on the Ilisu Dam that allow for fish migrations. For this reason, it is thought that there are potential changes in fish populations in the future, as fish migration to upper and lower basins is prevented. Identifying fish species prior to river impoundment not only provides important information on understanding of changes in the biotic communities of the river caused by dams, but also helps in designing strategies to conserve natural fish communities even in a changing ecosystem (Affonso et al., 2015).

Therefore, the purpose of this research was to determine the existing fish fauna including natives, endemics, exotics, and introduced species species from the main course of the Tigris River and its tributaries to be covered by the dam reservoir before construction of Ilisu Dam in southeast of Turkey. The information obtained will contribute to planning strategies for the conservation of fish species of Tigris River in Turkey.

MATERIAL AND METHODS

This study was carried out between 2010 and 2014 in the Ilisu reservoir of the Tigris River and its tributaries flowing into this area (Fig. 1). Fish specimens were collected with DC electrofishing device, cast net, and small gill nets in the shallow small streams. On the main course of the river, fishing nets suitable for river fishing (18, 24, 32, 45 and 60 mm porous and 50-100 m long) were used with a rubber boat during day and night.

The identification of the fish species was made according to Coad (2010) and Kaya et al (2016). The checklist is arranged by family following Van der Laan (2018). Valid species names, authorities, and year of publication follow Van der Laan et al (2014) and Fricke et al. (2021); genera and species within families are arranged in alphabetical order. English and Turkish names of the species were based on Ünlü (2014) and Jouladeh-Roudbar et al. (2020).

Red list categories are used as published by IUCN (2021). Species abundance has been revealed by making species identification and individual counts of each species in the field. The species caught were categorized in four groups according to their abundance status; year-round species (A), less common species (LA), rare species (R) and very rare species (VR). Their commercial importance has been derived from local fishermen. The coordinates of the areas monitored and the species detected were taken with a GPS.

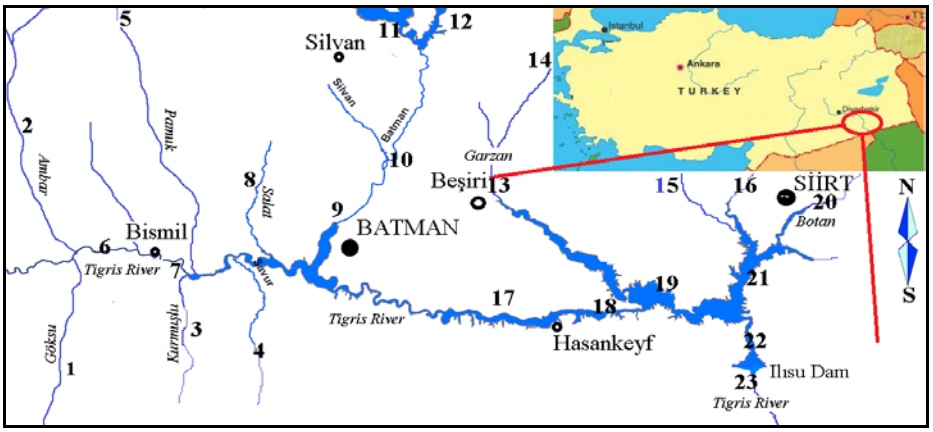

Figure 1: Map of the Ilisu Dam area on the Tigris River and sampled sites (Tab. 2).

RESULTS AND DISCUSSION

Lately, Kralkizi and Dicle Dams have been constructed on the main body of the Tigris River and put into operation for power generation and irrigation purposes respectively. Then Ilisu Dam started operation in 2019. Freshwater systems are highly threatened by massive changes in streams such as habitat loss, pollution, the emergence of non-native species, water consumption, dam construction, and require immediate action to ensure their protection for future generations (Collier et al., 1996; Cooper et al., 2016). In general, an intensive organic pollution is observed in the river as a result of agricultural activities in Bismil district and Batman province, which is the starting point of the Ilisu reservoir (Varol et al., 2010; Varol and Şen, 2012). At the same time, wild irrigation in this region leads to increased river sedimentation and heavy metals accumulation (Karadede-Akin and Ünlü, 2007; Varol, 2013). This situation causes adverse effects on fish species (Alrubayi et al., 2011).

In this study made before impoundment of the Ilisu Dam, 35 fish species belonging to 10 families were identified in the main course of the Tigris River, in the estimated reservoir area of the designed dam and in the tributaries joining this area (Tab. 1).

Table 1a: The natural, endemic, and exotic fish species in the main body of the river and the tributaries joining this area before impoundment of the Ilisu Dam; abundant (A), less abundant (LA), rare (R), and very rare (VR).

Family	Species	English name	Turkish local name	IUCN criteria	Status	Commercial value	Abundance
Cyprinidae	*Acanthobrama marmid* Heckel, 1843	Bream-Like.	Kızılkanat	LC	[N]	Medium	A
Cyprinidae	*Alburnus caeruleus* Heckel, 1843	Black spotted bleak	Benekli incibalığı	LC	[E]	Low	A
Cyprinidae	*Alburnus sellal* Heckel, 1843	Mossul bleak	Musul incibalığı	LC	[E]	Medium	A
Cyprinidae	*Arabibarbus grypus* Heckel, 1843)	Shabout	Şabot	VU	[N]	High	R
Cyprinidae	*Barbus lacerta* Heckel, 1843	Lizard barbel	Benekli bıyıklıbalık	LC	[N]	Medium	A
Cyprinidae	*Barilius mesopotamicus* Berg, 1932	Mesopotamian minnow	Mesopotamya minikbalığı	LC	[E]	Low	LA
Cyprinidae	*Capoeta trutta* (Heckel, 1843)	Long spine scraper	Berat	LC	[N]	High	A
Cyprinidae	*Capoeta umbla* (Heckel, 1843)	Tigris scraper	Şah	LC	[N]	High	A
Cyprinidae	*Carasobarbus kosswigi* (Ladiges, 1960)	Kiss–lip himri	Beyaz karagöz	VU	[E]	Low	VR
Cyprinidae	*Carasobarbus luteus* (Heckel, 1843)	Mesopotamian Himri	Himri, Karagöz	LC	[N]	High	A
Cyprinidae	*Carassius gibelio* (Bloch, 1782)	Prussian carp	Gibel sazanı	NE	[I]	High	A
Cyprinidae	*Chondrostoma regium* (Heckel, 1843)	King nase	Zereke, kababurun	LC	[N]	High	A
Cyprinidae	*Cyprinion kais* Heckel, 1843	Smallmouth lotak	Küçükağızlı bunni	LC	[E]	Medium	A
Cyprinidae	*Cyprinion macrostomus* Heckel, 1843	Largemouth lotak.	Bunni balığı	LC	[N]	Medium	A
Cyprinidae	*Cyprinus carpio* Linnaeus, 1758	Carp	Sazan	LC	[I]	High	A
Cyprinidae	*Garra rufa* (Heckel, 1843)	Common garra	Vantuzlu balık,	LC	[N]	Low	A
Cyprinidae	*Garra variabilis* (Heckel, 1843)	Smallmout garra	Yağlıbalık	LC	[N]	Low	A
Cyprinidae	*Leuciscus vorax* (Heckel, 1843)	Tigris asp	Sis balığı	LC	[E]	High	R
Cyprinidae	*Luciobarbus esocinus* Heckel, 1843	Mangar, Tigris barbel	Cero, Caner	VU	[E]	High	R
Cyprinidae	*Luciobarbus mystaceus* (Pallas, 1814)	Euphrates barbel	Nakkor, Sırink	NE	[E]	High	LA
Cyprinidae	*Luciobarbus subquincunciatus* (Günther, 1868)	Leopard barbel	Leopar sazan	CR	[E]	High	VR
Cyprinidae	*Squalius berak* Heckel, 1843	Mesopotamian chub	Tatlısu kefali	LC	[N]	Medium	A
Cyprinidae	*Squalius lepidus* Heckel, 1843	White chub	Kuzu, Akbalık	LC	[N]	High	A

Table 1b: The natural, endemic, and exotic fish species in the main body of the river and the tributaries joining this area before impoundment of the Ilisu Dam; abundant (A), less abundant (LA), rare (R), and very rare (VR). CR: (critically endangered): extremely high risk of extinction species in the wild. VU: (vulnerable): species that possess a very high risk of extinction as a result of rapid population declines. LC: (least concern): species that are pervasive and abundant after careful assessment. DD: (data deficient): Species with insufficient information on them. NE: (not evaluated): Species that have not been evaluated until now to meet the above criteria. [E]: Endemic species for Tigris and Euphrates Basin. [N]: Natural species. [I]: Alien introduced species

Family	Species	English name	Turkish local name	IUCN criteria	Status	Commercial value	Abundance
Mugilidae	*Planiliza abu* (Heckel, 1843)	Abu mullet	Dicle kefali	LC	[N]	High	A
Mastacembelidae	*Mastacembelus mastacembelus* (Banks and Solander, 1794)	Mesopotamian spiny eel	Mezopotamya yılanbalığı	LC	[N]	High	A
Sisoridae	*Glyptothorax steindachneri* (Pietschmann, 1913)	Euphrates sucking catfish	Vantuzlu kedibalığı	NE	[E]	Low	VR
Sisoridae	*Glyptothorax kurdistanicus* (Berg, 1931)	Mezopotamian sucking catfish	Mezopotamya vantuzlu kedibalığı	DD	[E]	Low	LA
Nemacheilidae	*Oxynoemacheilus kurdistanicus* Kamangar, Prokofiev, Ghaderi and Nalbant, 2014	Tigris loach	Tigris Çöpçü balığı	LC	[E]	Low	A
Nemacheilidae	*Oxynoemacheilus frenatus* (Heckel, 1843)	Tigris loach	Tigris Çöpçü balığı	LC	[E]	Low	A
Nemacheilidae	*Turcinoemacheilus kosswigi* Bănărescu and Nalbant, 1964	Zagros loach	Zagros çöpçübalığı	LC	[E]	Low	R
Siluridae	*Silurus triostegus* (Heckel, 1843)	Mezopotamian catfish	Mezopotamya yayını	LC	[E]	High	A
Heteropneustidae	*Heteropneustes fossilis* (Bloch, 1794)	Asian stinging catfish	Zehirli kedibalığı	LC	[I]	Low	R
Bagridae	*Mystus pelusius* (Solander, 1794)	Tigris mystus	Tahtakafa balığı	LC	[E]	Low	LA
Poeciliidae	*Gambusia holbrooki* Girard, 1859	Eastern mosquitofish	Sivrisinek balığı	LC	[I]	Low	A
Salmonidae	*Oncorhynchus mykiss* (Walbaum, 1792)	Rainbow trout	Gökkuşağı alabalığı	NE	[I]	High	R

Glyptothorax steindachneri caught from Tigris River is a new record for this area in Turkey (Fig. 2g).

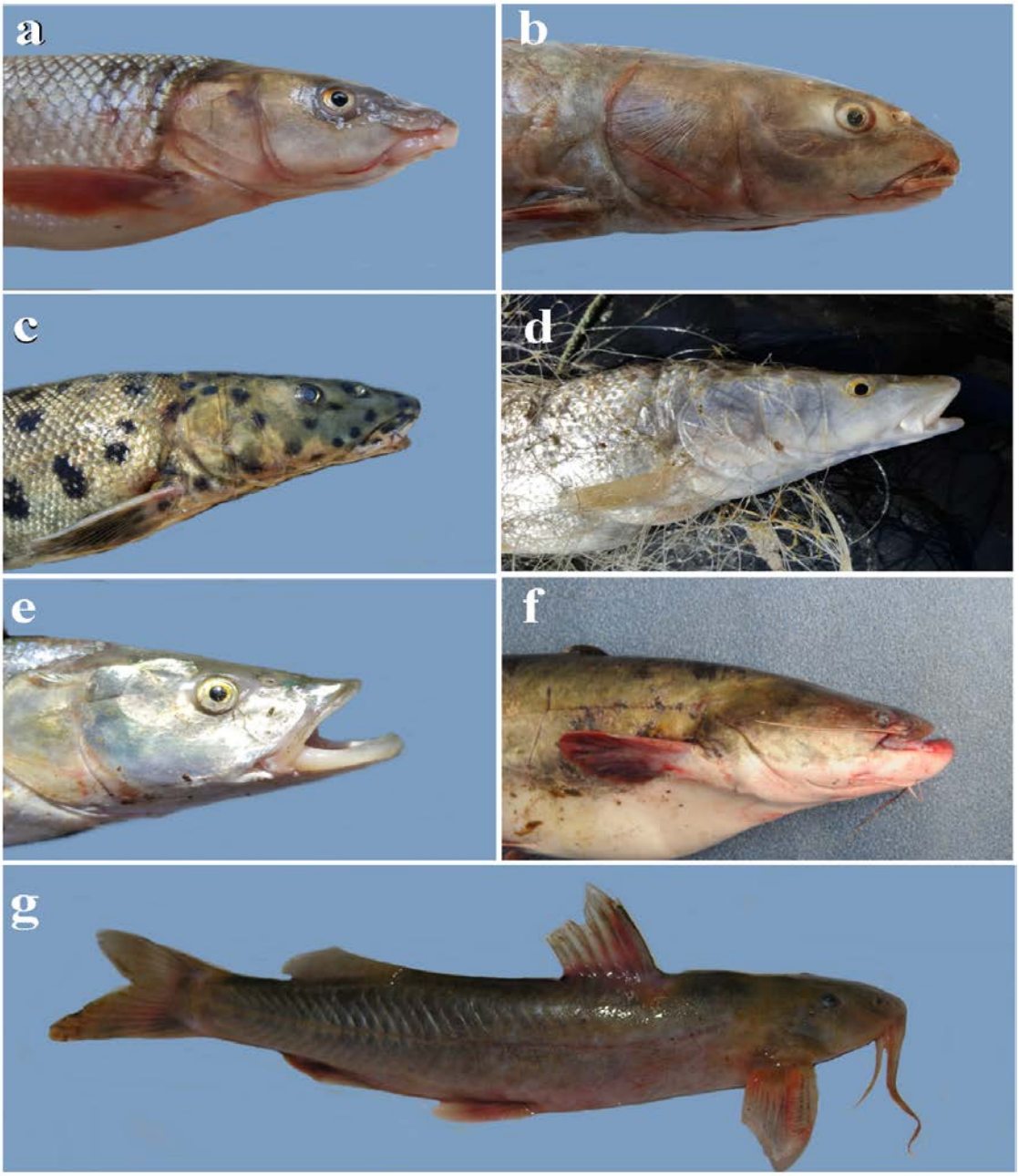

Figure 2: Some Cyprinid species collected in sampling sites. a, *Luciobarbus mystaceus* 502 mm; b, *Arabibarbus grypus* 545 mm; c, *Luciobarbus subquincunciatus*, 545 mm; d, *Luciobarbus esocinus*, 324 mm; e, *Leuciscus vorax* 532 mm; f, *Silurus triostegus* 650 mm; g, *Glyptothorax steindachneri* 18 mm.

22 species were found widely distributed. It is noted that the upper Tigris Basin exhibits rich fish fauna. In the studies conducted during 1970s, 28 species belonging to the eight family were reported (Kuru, 1975). In a recent study, this number was found to increase to 40 species belonging to 10 families (Kaya et al., 2016). The existence of 35 species presented in this study belonging to 10 families in the Ilisu Dam area is a testament to the importance of the fish biodiversity of this part of the river. In addition, the species *Leuciscus vorax*, *Luciobarbus esocinus*, *Luciobarbus subquincunciatus*, and *Carasobarbus kosswigi* are found only in this region of upper Tigris, whose populations have decreased considerably.

Distribution of fish species in the main course of the Tigris River and its tributaries before impoundment of Ilisu Dam according to sampling stations are given in table 2.

Table 2a: Distribution of fish species according to sampling stations in the Tigris River in relation to the Ilisu Dam.

Station No.	Locality	Species
1.	Çınar district Göksu Stream 37°41’21.76’’N 40°26’50.42’’, 668 m	*Alburnus sellal, Capoeta trutta, Capoeta umbla, Carassius gibelio, Garra variabilis, Squalius berak, Oxynoemacheilus kurdistanicus*, and *Gambusia holbrooki.*
2.	Ambar Stream, 37°56’40.91’’N 40°25’21.36’’E, 621 m	*Acanthobrama marmid, Alburnus caeruleus, Alburnus sellal, Capoeta trutta, Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Garra variabilis, Squalius berak, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Oxynoemacheilus kurdistanicus, Oxynoemacheilus frenatus*, and *Gambusia holbrooki.*
3.	Kurmuşlu Stream before Bismil Tepe Town 37°46’35.52’’N 40°47'56.82”E, 566 m	*Acanthobrama marmid, Alburnus caeruleus, Alburnus sellal, Barbus lacerta, Capoeta umbla, Carasobarbus kosswigi, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Garra rufa, Squalius berak, Oxynoemacheilus kurdistanicus, Oxynoemacheilus frenatus, Turcinoemacheilus kosswigi, Gambusia holbrooki*, and *Oncorhynchus mykiss*.
4.	Savur Stream after Tepe Village 37°47’25.75’’N 40°52’30.25’’E, 559 m	*Alburnus sellal, Barbus lacerta, Capoeta umbla, Carasobarbus kosswigi, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Garra rufa, Squalius berak, Oxynoemacheilus kurdistanicus, Oxynoemacheilus frenatus, Turcinoemacheilus kosswigi*, and *Oncorhynchus mykiss.*
5.	Pamuk Stream 38°5’47.64’’N 40°35’38.99’’E, 717 m	*Acanthobrama marmid, Alburnus caeruleus, Alburnus sellal, Barbus lacerta, Barilius mesopotamicus, Capoeta trutta, Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Luciobarbus mystaceus, Squalius berak, Squalius lepidus, Planiliza abu, Oxynoemacheilus kurdistanicus, Oxynoemacheilus frenatus*, and *Turcinoemacheilus kosswigi.*

Table 2b: Distribution of fish species according to sampling stations in the Tigris River in relation to the Ilisu Dam.

Station No.	Locality	Species
6.	Tigris River, before Bismil, 37°50'52.07''N 40°36'10.57'E, 546 m	*Acanthobrama marmid, Alburnus caeruleus, Barilius mesopotamicus, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus esocinus, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Silurus triostegus, Heteropneustes fossilis*, and *Gambusia holbrooki.*
7.	Tigris River after Bismil Town (Ilisu Dam lake initial zone) 37°49'15.09''N 40°49'18.51''E, 530 m	*Acanthobrama marmid, Alburnus caeruleus, Barilius mesopotamicus, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus esocinus, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Silurus triostegus, Mystus pelusius*, and *Gambusia holbrooki.*
8.	Salat Stream, 37°52'39.67''N 40°52'7.91''E, 540 m	*Acanthobrama marmid, Alburnus caeruleus, Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Squalius berak, Planiliza abu, Mastacembelus mastacembelus, Oxynoemacheilus kurdistanicus*, and *Gambusia holbrooki.*
9.	Batman Stream, Batman 37°53'24.37''N 41°2'36.43''E, 530 m	*Alburnus sellal, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Glyptothorax kurdistanicus*, and *Oxynoemacheilus kurdistanicus.*
10.	Malabadi Bridge, 38°9'7.80''N 41°12'24.98''E, 604 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Glyptothorax kurdistanicus*, and *Mystus pelusius.*
11.	Batman reservuar, 38°12'42.82''N 41°8'31.94''E, 657 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Squalius lepidus, Planiliza abu*, and *Mystus pelusius.*
12.	Sason Stream, 38°16'38.41''N 41°11'5.31''E, 673 m	*Acanthobrama marmid, Alburnus caeruleus, Alburnus sellal, Barbus lacerta, Barilius mesopotamicus, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Garra rufa, Squalius berak, Glyptothorax kurdistanicus, Oxynoemacheilus kurdistanicus, Oxynoemacheilus frenatus.*
13.	Garzan River, Beşiri Town 37°53'56.31''N 41°22'4.57''E, 530 m	*Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Squalius berak, Glyptothorax kurdistanicus*, and *Oxynoemacheilus kurdistanicus.*

Table 2c: Distribution of fish species according to sampling stations in the Tigris River in relation to the Ilisu Dam.

Station No.	Locality	Species
14.	Garzan Stream (Kozluk), 38°9'34.86''N, 41°30'47.70''E, 640 m	*Alburnus sellal, Capoeta trutta, Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Luciobarbus mystaceus, Squalius berak, Planiliza abu,* and *Mastacembelus mastacembelus.*
15.	Başur Stream (Baykan), 37°57'50.60''N 41°47'22.59''E, 521 m	*Capoeta umbla, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Squalius berak, Glyptothorax kurdistanicus,* and *Oxynoemacheilus kurdistanicus.*
16.	Kayser Stream, 37°56'50.15''N 41°51'14.92''E, 530 m	*Acanthobrama marmid, Alburnus sellal, Capoeta trutta, Capoeta umbla, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Garra rufa, Squalius berak, Oxynoemacheilus kurdistanicus,* and *Oxynoemacheilus frenatus.*
17.	Tigris River, Suçeken Village, Hasankeyf, 37°44'15.04''N 41°17'17.00''E, 505 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Glyptothorax steindachneri, Glyptothorax kurdistanicus, Silurus triostegus, Heteropneustes fossilis,* and *Mystus pelusius.*
18.	Tigris River, after Hasankeyf, 37°43'54.63''N 41°30'20.52''E, 501 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Glyptothorax steindachneri, Glyptothorax kurdistanicus, Silurus triostegus, Heteropneustes fossilis,* and *Mystus pelusius.*
19.	Junction area of Tigris River and Garzan Stream, 37°43'58.19''N 41°37'6.51''E, 480 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Glyptothorax steindachneri, Glyptothorax kurdistanicus, Silurus triostegus, Heteropneustes fossilis,* and *Mystus pelusius.*
20.	Botan River, Siirt 37°53'22.78''N 41°56'40.16''E, 480 m	*Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Leuciscus vorax, Luciobarbus esocinus, Luciobarbus mystaceus,* and *Squalius lepidus.*

Table 2d: Distribution of fish species according to sampling stations in the Tigris River in relation to the Ilisu Dam.

Station No.	Locality	Species
21.	Tigris River and Botan Stream confluence area 37°42'50.86''N 41°48'29.43'', 436 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion macrostomus, Cyprinus carpio, Garra variabilis, Luciobarbus mystaceus, Squalius lepidus, Planiliza abu, Mastacembelus mastacembelus, Glyptothorax steindachneri, Glyptothorax kurdistanicus, Silurus triostegus, Heteropneustes fossilis,* and *Mystus pelusius.*
22.	Tigris River, before Ilisu Dam, Güçlükonak-Siirt road, 37°36'32.10''N 41°52'1.89''E, 436 m	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Cyprinion macrostomus, Cyprinus carpio, Garra rufa, Leuciscus vorax, Luciobarbus esocinus, Luciobarbus mystaceus, Luciobarbus subquincunciatus, Squalius lepidus, Planiliza abu, Glyptothorax steindachneri, Glyptothorax kurdistanicus*, and *Mystus pelusius.*
23.	Tigris River, after diversion tunnels of Ilisu Dam 37°31'18.43''N 41°50'38.99''E, 420 m;	*Acanthobrama marmid, Alburnus sellal, Arabibarbus grypus, Capoeta trutta, Capoeta umbla, Carasobarbus kosswigi, Carasobarbus luteus, Carassius gibelio, Chondrostoma regium, Cyprinion kais, Cyprinion macrostomus, Cyprinus carpio, Garra rufa, Leuciscus vorax, Luciobarbus esocinus, Luciobarbus mystaceus, Luciobarbus subquincunciatus, Squalius lepidus, Planiliza abu, Glyptothorax steindachneri, Glyptothorax kurdistanicus*, and *Mystus pelusius.*

This study recorded 22 species as abundant and among them *Acanthobrama marmid*, *Alburnus sellal*, *Carassius gibelio*, *Cyprinion macrostomus*, and *Chondrostoma regium* were the most common species. This was followed by less abundant species *Capoeta umbla*, *Capoeta trutta*, *Chondrostoma regium, Barilius mesopotamicus*, *Luciobarbus mystaceus*, *Glyptothorax kurdistanicus*, and *Mystus pelusius*. Availability of *Arabibarbus grypus*, *Leuciscus vorax*, *Luciobarbus esocinus*, and *Turcinoemacheilus kosswigi* were found as rare while availability of *Carasobarbus kosswigi*, *Luciobarbus subquincunciatus*, and *Glyptothorax steindachneri* were found as very rare.

The most diverse family recorded in this study was the Cyprinidae with 23 confirmed species (65.7%) (Fig. 3). Five of the species (*Carassius gibelio, Cyprinus carpio, Heteropneustes fossilis, Oncorhynchus mykiss*, and *Gambusia holbrooki*) are exotic. Among these, *Carassius gibelio* and *Gambusia holbrooki* are also invasive species appeared throughout the river.

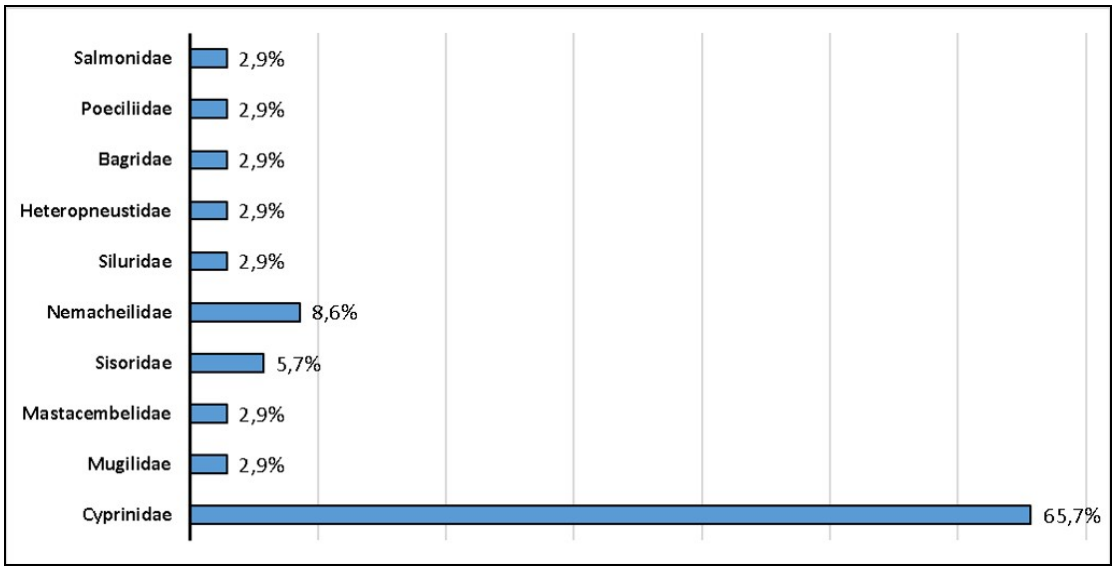

Figure 3: Percentage of species according to family in Ilisu area.

In the area of impact of the Ilisu Dam, at least 16 species were recorded, which are endemic to the Tigris and Euphrat basins (Fig. 4). Furthermore, three species were already recommended with some level of international protection on the IUCN Red List (2021). These species are *Carasobarbus kosswigi* (vulnerable), *Luciobarbus esocinus* (vulnerable), *Luciobarbus subquincunciatus* (critically endangered) (Fig. 5). *Cobitis kellei* and *Paraschistura chrysicristinae* species, whose type localities are reported as upper Tigris River, have not been observed in this tudy.

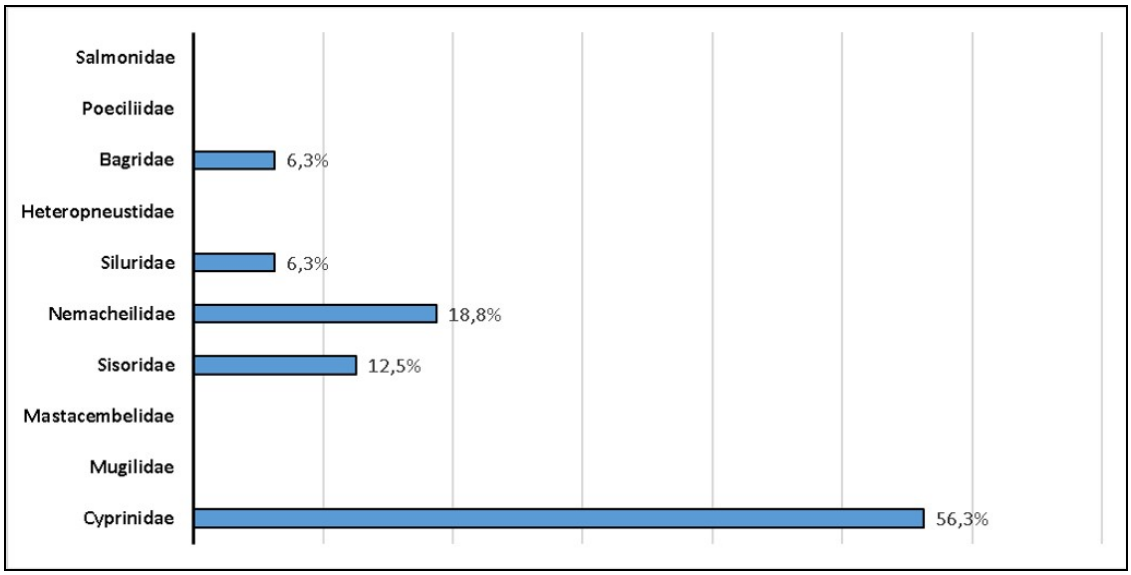

Figure 4: Percentage of endemics according to family in Ilisu area.

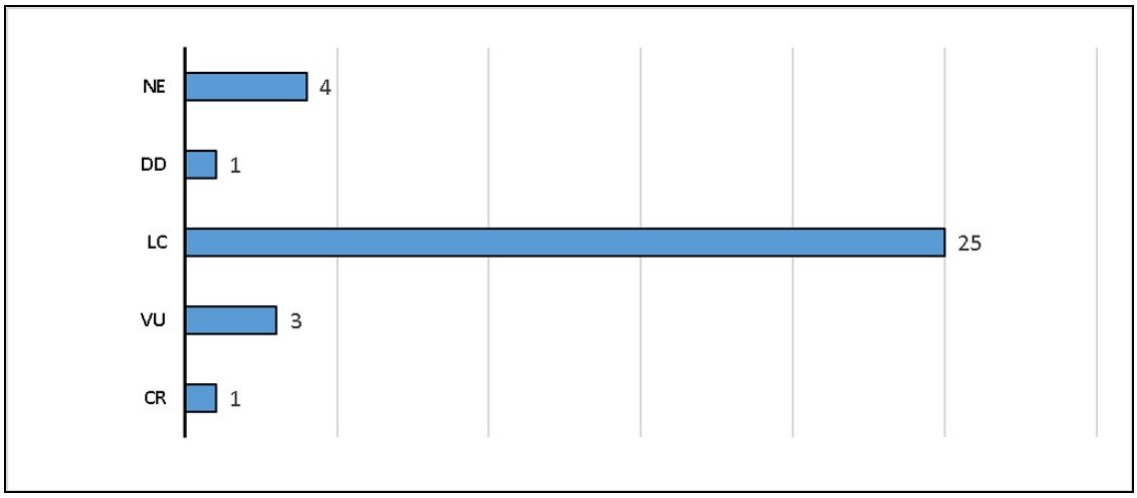

Figure 5: Distribution of species in Ilisu area according to IUCN criteria. CR (critically endangered, VU (vulnerable), LC (least concern), DD (data deficient), NE (not evaluated).

Leuciscus vorax (Heckel, 1843) was described from Tigris River (Mossul) and it was obtained only in the downstream part of the Ilisu Dam. Population of this species has decreased in Tigris River but is still assessed on level of LC of the IUCN Red List (2021). We recommend that it should be included in protected species list as VU.

Cobitis kellei reported from Goksu Stream by Erk'akan et al. (1998) and *Paraschistura chrysicristinae* from Batman Stream by Nalbant (1998) could not be obtained in the present study. Similar situation was also reported by Kaya et al. (2016).

Glyptothorax steindachneri is a little known species from Turkey, only recorded from Euphrates at Kemaliye (Freyhof et al., 2021). In the present study, this species is reported for the first time from the Turkish part of the Tigris River.

Although *Luciobarbus subquincunciatus* and *Luciobarbus esocinus* species was recorded in previous studies (Kuru, 1978; Kaya et al., 2016), it has been obtained very rarely and only in a very limited area of Ilisu Dam, due to overfishing and excessive pollution of the river and subsequent decrease of its population in recent years.

CONCLUSIONS

Large dams and water pollution caused by intensive agricultural activities in the region cause negative effects on aquatic creatures living in the rivers built for electricity and irrigation purposes. In order to minimize these effects and protect the aquatic ecosystem, it should be one of the main objectives to identify the fish fauna of the environment in advance and reveal the species that may be affected by this change. Thus, this study was carried out before the construction of the Ilisu Dam and fish species were determined. Many of the fish species in the Tigris River have adapted to living in the flowing waters of the river ecosystem, and it is recommended that at least the tributaries joining the rivers be streamlined.

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COŞTEI HYDROGRAPHIC DIVERSION NODE, A HISTORICAL ENVIRONMENT QUALITY AND BIOLOGICAL RESOURCES ACCESSIBILITY GAME CHANGER; ANTHROPOGENIC INDUCED PROBLEMS AND SUSTAINABLE SOLUTIONS – AN ICHTHYOLOGIC PERSPECTIVE

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KEYWORDS: lotic system, hydrotechnical diversion work, habitat change, biological/fish resources loss, bio-eco-economy, sustainable connectivity reconstruction.

ABSTRACT

26 fish species were affected by the Coştei historical diversion hydrotechnical system build in 1758. In order to mitigate the negative effects produced by this hydrotechnical work on the fish, a migration system, of nature-like meandering by pass type was proposed. The dimensions of this channel and the slope of about 2% allow fish, and other aquatic organisms to move upstream and downstream of the spillway.

RÉSUMÉ: Le nœud de dérivation hydrographique de Coştei, une qualité historique de l'environnement et l'accessibilité aux ressources biologiques changent la donne; problèmes anthropiques induits et solutions durables – une perspective ichtyologique.

26 espèces de poissons ont été affectées par le Coştei système hydrotechnique de dérivation historique construit en 1758. Afin d'atténuer les effets négatifs produits par ces travaux hydrotechniques sur les poissons, un système de migration, de méandres par type de passe, de nature similaire a été proposé. Les dimensions de ce chenal et la pente d'environ 2% permettent aux poissons et à d'autres organismes aquatiques de se déplacer en amont et en aval du déversoir.

REZUMAT: Nodul hidrografic deversor Coştei, un modificator istoric al calităţii mediului şi a accesibilităţii resurselor biologice; probleme induse antropic şi soluţii durabile – o perspectivă ihtiologică.

26 specii de peşti au fost afectate de nodul hidrotehnic deversor Coştei construit în 1758. Pentru a atenua efectele negative produse de această lucrare hidrotehnică asupra peştilor, s-a propus un sistem de migraţie, de tip by pass meandrat. Dimensiunile acestui canal şi panta de aproximativ 2% permit peştilor şi altor organisme acvatice să se deplaseze în amonte şi în aval de deversor.

INTRODUCTION

Over one billion of people are suffering by hunger and over one billion by malnutrition, the accessibility to animal and especially high quality animal protein (i.e. fish protein) being one of the key problem (Bănăduc et al., 2012a). The bio-economy and eco-economy aim to combat the fish protein scarcity (Bănăduc et al., 2012b). There are many causes with important global effects that reveal the decision makers the value of sustainable development as a foundation for bio-eco-economy, based on science which offers solutions for a wise use of natural resources and services, and avoid and/or diminish the local, regional, and global ecological potential support overshoot. Among these causes are: the last economic crisis that shook all national economies and international capital markets (Lane, 2010; Porfirev, 2010); inflation that affects even efficient and stable economies (Campbel et al., 2009; Che et al. 2011), accelerated and continuous growth of the human population (Bongaarts, 2009; Lutz, 2011), migration dynamics (Debra, 2001; Solano-Garcia, 2009), the decreasing level of education (Mok, 2010), unsatisfactory health of human population (Morris, 2009; Samir, 2009), the occurrence at irregular intervals of natural and/or anthropogenic induced disasters (Berger, 2010; Land, 2010), deterioration of the qualitative and quantitative characteristics of some ecological support systems and of the resources and/or services offered by them to the human society (Curtean-Bănăduc et al., 2007), the need for biofuels (Bogdan et al., 2010), hunger or high food prices in some parts of the world (Maneschi et al., 1997; Brinkman et al., 2010), climate changes and disruption (Boston and Lempp, 2011; Tamura, 2011), biodiversity loss and the sixth mass extinction (Barnosky et al., 2011; Bradshaw et al., 2021), the security level decreasing and negative economic effects induced by the actions of terrorist entities that are hostile to the currently international status quo (Pan et al., 2009; Sela-Shayovitz, 2010), political impotence (Hejny, 2018; *, 2018), etc.

Water as one of the most essential natural resources and habitat is not only an "elementary" fundamental source for life on Earth, but also has an essential importance on the environmental, economic, and social equilibrium and development of the world (Falkenmark, 2020; Zhang et al., 2021). Water natural resources are the most exceedingly important element of the environment liable for the development of human civilization (Juuti et al., 2007; Cianfaglione, 2018), for sustainable development (Unesco Water, 2006; Oprean, 2012), and turn into a vital issue in the present climate change reality (Şahin et al., 2020), and other global changes (Cianfaglione, 2014; Gulbenkian Think Tank, 2014). The water quantity and quality of freshwater systems, descending resources, is turning into a significant problem for almost two-thirds of the all-encompassing human population; almost all the regional scale water basin faces serious water quality and quantity issues and their corresponding resources insufficiency or/and inadequacy (Abu-Zeid and Shiklomanov, 2004; Mekonnen and Hoekstra, 2016). Freshwater systems in spite of their importance, in this regard, have declined more rapidly than marine habitats (Sala et al., 2000). Even though enclosing barely 0.8% of our planet surface, fresh waters have one-third of the named vertebrate species and 9.5% of the known animal species, and around 50% of all fish species (Joy et al., 2019). The health of lotic aquatic ecosystems degraded physically, chemically, and biologically due to wide variety of threats (climate change, pollution, water abstraction, river channelization, damming). Lotic systems are among the most degraded ecosystems of the Earth (Vörösmarty et al., 2010).

The most diverse lotic systems' on earth are highly threatened due to river fragmentation (Bosshard, 2015). A serious threat that influences aquatic communities is longitudinal fragmentation (Birk et al., 2012). For example, the building of regulators, dams, and weirs for various purposes (e.g., municipal water supply, flood control, irrigation, hydropower, navigation) change water temperature, movement of water and sediment, and

exchange of nutrients, which adversely impact the environmental flow requirements (Acreman and Dunbar, 2004) and, afterward, hamper fish populations and other aquatic organisms (Soolutayo, 2012; Olopade, 2013; Rumana et al., 2015). In the present anthrophosphere, the water, the aquatic and semi-aquatic habitats and associated non-living and living elements are considered and treated as resources, being overused, reshaped, and changed by humans (Barinova and Krassilov, 2012; Del Monte-Luna et al., 2016; Sender et al., 2017; Lemenkova 2020). Recognizing the progressing habitat fragmentation worldwide, research put effort into conservation measures for restoring connectivity of riverine habitats (Branco et al., 2014).

In the last decade, some improvement has been made in recover the rivers and streams longitudinal connectivity by implementing ecological principles including in the studied Carpathian Basin area (Voicu and Voicu, 2014, 2015; Bănăduc et al., 2020a; Voicu et al., 2020). Although, there are some local solutions for restoring longitudinal connectivity in the Timiş Basin (Curtean-Bănăduc et al., 2018), remains one of the unresolved challenge of completely restoring this connectivity due to the attenuation of hydromorphological pressures generated by transversal hydrotechnical works, not only in the Timiş River basin but worldwide (Fuller et al., 2015). Based on the European Water Framework Directive, the ecological capabilities of bypass canals is an urgent need for ecological improvement of rivers (Pander, 2013). Creating an alternative depth bypass increases the efficiency of the bypass compared to the existing surface bypass (Knott et al., 2019).

The Danube River's 801,093 km^2 basin, to which the Timiş Basin belongs, is not a different case, the most ancient known human footprint date since 180.000 B.C., being one of the most relevant European watersheds from the points of view of its natural environment, history, culture, and economic value (Liepolt, 1967; Tockner et al., 2009; Bănăduc et al., 2016). The Danube basin is adversely influenced by a diversity of human-made impact effects: habitats fragmentation and isolation (Popa et al., 2019); ecosystem degradation (Popa et al., 2016); water, sediments, and fish pollution (Bănăduc et al., 2011; Curtean-Bănăduc et al., 2020b); decreasing fisheries production of economically fish species and hybridization (Popa et al., 2017); riverine land exploitation (Curtean-Bănăduc et al., 2007); riverine ecotone degradation (Curtean-Bănăduc et al., 2019); disruption of water and sediment flow (Curtean-Bănăduc et al., 2018); with increasing negative effects due to climate change (Bănăduc et al., 2020b), all of these bringing in light the need for special management measures for the Danube River basin (Bloesch et al., 2012; Bănăduc et al., 2021). Past and present anthropogenic environmental stress reflects the high susceptibility of natural freshwater ecosystems in the lower Danube basin too (Iordache et al., 2020). As a part of the Danube middle-lower basin, the Timiş River has fluvial related characteristics (stream networks types, hydro-geomorphologic structures, elevation, landforms, etc.) which were from ancient time determinant attractants for constructions and human settlements (Petrescu and Hosu, 2020).

The historical human pressures that have a negative impact on the Timiş River basin are hydro-technical works, water accumulations, settlement works and construction of dikes and banks defence, agricultural and industrial development, and urbanization (Burghelea et al., 2013). Between 1728 and 1732, a significant part of the river Bega course was regularized, creating in its lower sector a 115 km navigable canal to support the Timișoara (the capital of Banat Region) growing economy. Thus, the city was connected, via the river Tisza and the Danube, to the Central European rivers and the Black Sea, becoming able to cope with mass transport before the advent of the railway. Therefore, the regularization of the Bega and Timiș courses changed the image of the city and surrounding area into a shining prosperous one.

Coştei hydrotechnical node is a diversion system located (45º44'10.88"N – 21º51'3.83") at the branch of the Timiş-Bega discharge channel from the Timiş River, in the west of Romania (Fig. 1). A dam house (Fig. 2) has been placed on this canal which aims to control the flow of water which is directed towards the Bega River/Canal (Fig. 3).

Figure 1: Coştei hydrotechnical diversion system area.

Figure 2: The flow of water control dam house.

Figure 3: The Bega River/Canal downstream the Coştei dam house.

This hydrotechnical node was built in 1758. Its purpose was and still is to continuously direct the waters of the Timiş River in Bega Canal especially during times of drought to maintain a significant higher and constant flow of water in the Bega River. Over 10,000 years old human settlement, energetic, complex, and continuous socio-economic development of Timişoara, the third biggest present Romanian city which maybe not by chance takes the name of Timiş River instead of Bega River which crosses it, and its metropolitan area with a total of over a million people in present, and the vast agriculture surroundings which need irrigations, create a continuous increasing thirst of a higher amount of water, downstream Coştei diversion system the Timiş River becoming much, much scarcer in water along history downstream Coştei River (Figs. 4-6).

Figure 4: Timiş River upstream the Coştei hydrotechnical diversion system.

This hydrotechnical node is composed of a spillway dam with an upper length of 877 m, width 127, and a height of 10.5 m, (Fig. 5) located on the Timiş River and Bega Canal (Fig. 3) that starts from Timiş upstream of the spillway dam.

Figure 5: Coştei Dam on Timiş River; no water was left to flow over the dam.

Figure 6: Timiş riverbed with scarce water flow downstream the Coştei Dam.

The Coştei Dam is an overflow dam with a wide threshold of boulders in wooden houses filled with raw stone. To avoid floods in Timişoara, another hydrotechnical system at Topolovățu Mic, during periods of high water, directs the excess flow recorded by Bega River back in the Timiș River. The Coştei and Topolovăţu Mic hydrotechnical works and the unnatural/human managed water flow through a series of barrages and deviation of water from one basin to another, like in the biggest effluents (Bistra-Sebeş basins) cases on the upper Timiş River course (Curtean-Bănăduc et al., 2018), create effects of unnatural flushing of fishes over the Coştei Barrage or in the Bega Canal system, without the possibility of recolonization upstream even in critical/reproduction periods, mainly due to a not ecological flow management and the lack of fish ladders or passages.

Since significant fish fauna changes were highlighted in the upper Timiş River basin (Bănăduc et al., 2013; Curtean-Bănăduc et al., 2018), a natural continuity of these studies carry on in its middle curse through this specific research related to the Coştei hydrotechnical work effects on the fish resources. This approach in identifying historical significant negative effects of such a major hydrographical node, after this massive diversion scheme was finished, and in situ adapted mitigation technical optimum solution, is a new and innovative one in the studied region.

To enhance the aquatic ecosystems, we aim attention to the temporal and spatial structural modifications of fish fauna, brought about by a hydrotechnical diversion system construction and management, on a medium sized lotic system (Timiş River, Danube Basin). Here at first, the impact and magnitude of this historic diversion system was assessed based on the fish fauna capacity to indicate changes in the ecological status of a lotic system. Then the authors proposed a conceptual design for sustainable in situ adapted technical solution (by-pass) and specific management measures, to mitigate these effects on the local and regional fish fauna.

MATERIAL AND METHODS

In general due to the fact that the studied hydrotechnical node was built back in 1758, the same year when Linnaeus determined and named the studied fish of this paper too, it is impossible to make a comparison of fish fauna structure since that year, due to the taxonomical and scientific related names lacking assciated problems. The data concerning the historical impact of this major hydrotechnical diversion system's effects on the aquatic ecosystem's ichthyofauna is scarce in the area of interest, the reason for which this study was done based on the accessible period fiserman intervievs data (Annex 1/Table 1), and up-to-date field research information (Annex 2/Table 2). The main targets of this analysis were to establish the role of this significant hydrotechnical diversion system on the local and regional lotic ichthyofauna temporal and spatial transformation, and to design and suggest an in situ suitable alleviation technical work for the recovery of the identified ecological and economical losses. The studied old diversion system impact magnitude was assessed based on the fish fauna capacity to indicate changes in the ecological status of a lotic system. Fishes are key elements of aquatic ecosystems and are key components of ecosystem services in both qualitative and quantitative aspects, through the high number of taxa and their variety of ecological needs, adaptations, and functions (Nelson, 1995; Day, 2006), these making them ideal indicators for aquatic environment changes (López-López et al., 2015; Levin et al., 2019).

All the available reliable scientific information, discussions with old fishermen in the area, present fisherman captures analysis and fish identifications in upstream and downstream river sectors, were analyzed and compared in the last decades in the study area.

Based on the past and present fish species in the research area and current biological and ecological needs, an innovative in situ adapted technical solution (by-pass) for fish fauna rehabilitation was designed. This by-pass channel is based on an appropriate model of ecosystem response and the recovery of the aquatic community.

RESULTS

The decreasing trend of some fish species of economic interest in the Coştei hydrotechnical work area in the last century

The first ichthyologic systematic great Romanian book about fish fauna of Antipa (Antipa, 1909) included some small parts of the Banat region, and the first reliable complete Romanian book regarding the fish fauna including the Timiş River basin was published due to the greatest Romanian ichthyologist masterpiece (Bănărescu, 1964). Supplementary historic local data about fish were obtained in the past from the first author grand-grandfather (Agăsân Andraş, 1905-1985), who was for a lifetime in the area of interest a peasant-fisherman, and from one of the most active and experienced game fishermen family in the area of interest Adam Josef and his son Adam Helmut Johann since 1945 till the present (Annex 1/Table 1). Based on these information sources, it was possible for a spatio-temporal comparison of the fish fauna changes with present day fish data obtained for this research in the last decades on the field by the authors (Annex 2/Table 2). Due to the lack of very old reliable data, we cannot cover all the period since 1758 after the construction/deviation was made, but we cover the later period when the water demand by modern Timişoara city area became significant bigger, and when started new consolidation and elevation works at the diversion system (the 80's till 2004). As a result we identified an obvious decreasing trend in terms of many fish species for this period.

Silurus glanis Linnaeus, 1758 (Actinopterygii, Siluriformes, Siluridae) is freshwater, brackish, benthopelagic, non-migratory, and autochthonous fish species in the Danube Basin area with a maximum published weight of 306 kg (Antipa 1909; Bănărescu, 1964; Frimodt 1995; Oţel 2007; Bănăduc et al., 2015). This species natural distribution covers Europe and Asia, North, Baltic, Caspian, and Aral seas basins, as far north as southern Sweden and Finland; Aegean Sea basin in Maritza and from the Struma to Sperchios drainages, Turkey, was introduced in Europe and Lake Balkhash area in Kazakhstan (Frimodt, 1995; Kottelat and Freyhof, 2007).

The wels catfish has a high direct important economic value and a high nutritional value. The meat has a pleasant, fatty taste, it is eaten fresh in all its forms (Antipa, 1909; Frimodt, 1995).

Since the wells catfish is a very important economic fish species in freshwater fishing, has an accentuated continuous decreasing trend both in the upstream and the downstream of Coştei hydrotechnical work it represents a first big loss for the local economy. In addition to its direct economic value, this species has also a conservative value, being protected by the Berne Convention.

Cyprinus carpio Linnaeus, 1758 (Actinopterygii, Cypriniformes, Cyprinidae) is freshwater, brackish, benthopelagic, potamodromous, autochthonous fish species in the Danube Basin, with a published maximum weight of 40.1 kg (Antipa, 1909; Frimodt, 1995; Kottelat 1997; Oţel, 2007; Levin, 2019). Its range including wild populations cover Europe to Asia: Black, Caspian, and Aral seas basins, was introduced all over the world (Kottelat and Freyhof, 2007). A reophilic natural population in the Danube is accepted to be the ancestor group of the European species, now under threat (Machacek, 2007).

The common carp is the ultimate economically important freshwater fish species of Europe and in the temperate climatic zone. This exceptionally important commercial and game fish species in Romania too is the principal target for fishing in the lower sectors of medium and large lotic systems with a sweet meat, somewhat fat; being eat up in all imaginable ways: soup, fried, brine, baked, stuffed, salted, smoked, caviar salad, etc. (Antipa, 1909; Frimodt, 1995).

Since this fish species has an outstanding importance in freshwater fishing and aquaculture, its identified radical decreasing trend in both the upstream and downstream of river sector of Coştei hydrotechnical work represents a second big loss for the local economy. The natural form of this fish species is also of conservative interest not only of economic interest, being included in the IUCN Red List, as vulnerable (VU) (Kottelat and Freyhof, 2007).

Lota lota (Linnaeus, 1758) (Actinopterygii, Gadiformes, Lotidae) is freshwater, brackish, demersal, potamodromous, autochthonous fish species in the Danube Basin, with a maximum published weight of 34 kg (Antipa, 1909; Morrow, 1980; Frimodt, 1995; Oţel, 2007; Levin et al., 2019). Its natural distribution cover circumarctic area: Europe Loire drainage, Barents and Arctic Sea basins; upper Volga drainage; western Caspian basin; rivers draining to Black Sea; Rhône drainage (France); in Italy native only in Po drainage. In Siberia eastward to River Lena. North America: throughout Canada, Alaska and northern USA (south to Kentucky, Missouri, Wyoming and Washington (Page, 2011).

The burbot is an important economic species in freshwater fishing and has a profound decreasing trend in both the upstream and downstream of Coştei it represents a third loss for the local economy (Bănăduc et al., 2014).

Sander lucioperca (Linnaeus, 1758) (Actinopterygii, Perciformes, Percidae, Luciopercinae) is freshwater, brackish, pelagic, potamodromous, and autochthonous fish species in the Danube Basin area, with a maximum published weight of 20 kg (Antipa, 1909; Frimodt 1995; Oţel, 2007; Keith and Allardi, 2002; Levin and Woodford, 2019). Its natural distribution covers both Europe and Asia: Caspian, Baltic, Black, and Aral Sea basins; Elbe (North Sea basin) and Maritza (Aegean basin) drainages. North to about 65° N in Finland (Welcomme, 1988). The pike-perch is an important economic fish species in freshwater fishing and has a significant decreasing trend in both the upstream and downstream of Coştei hydrotechnical work area, it represents a fourth loss for the local economy (Bănăduc et al., 2014).

Tinca tinca (Linnaeus, 1758), (Actinopterygii, Cypriniformes, Cyprinidae, Tincinae) is freshwater, brackish, demersal, autochthonous fish species in the Danube Basin, with a maximum published weight of 7.5 kg (Antipa, 1909; Muus and Dahlström 1968, Frimodt 1995; Oţel 2007; Levin et al., 2019). Its natural distribution covers Eurasia: hypothesized as native in most Europe, in Asia native eastward to western Yenisei drainage (Kottelat and Freyhof, 2007).

The rudd is an important economic fish species in freshwater fishing and has an accentuated decreasing trend in both the upstream and downstream of Coştei hydrotechnical work it represents a fifth loss for the local economy.

Abramis brama (Linnaeus, 1758) (Actinopterygii, Cypriniformes, Cyprinidae, Leuciscinae) is freshwater, brackish, benthopelagic, autochthonous fish species in the Danube Basin, with a maximum published weight of six kg (Antipa, 1909; Bănărescu 1964; IGFA 1991; Oţel 2007; Bănăduc et al., 2014). Its natural distribution covers Europe and Asia: most European drainages from Adour (France) to Pechora (White Sea Basin), Aegean Sea basin, in Lake Volvi, and Struma and Maritza drainages. In Asia, from the Marmara Basin (Turkey) and eastward to the Aral Basin (Kottelat and Freyhof, 2007).

Since the freshwater bream is an important economic fish species in freshwater fishing, and has a high and accentuated continuous decreasing trend in both in the upstream and the downstream of the upstream Coştei hydrotechnical work it represents a sixth big loss for the local economy.

Rutilus rutilus (Linnaeus, 1758), (Actinopterygii, Cypriniformes, Cyprinidae, Leuciscine) is freshwater, brackish, benthopelagic, autochthonous fish species in the Danube Basin, with a maximum published weight of 1.8 kg (Antipa, 1909; Bănărescu 1964; IGFA 1991; Oţel, 2007; Bănăduc et al., 2014). Its natural distribution covers Europe: north to Pyrenees and Alps, eastward to Ural and Eya drainages (Caspian Basin); Aegean Basin in Pinios, Vardar, Vegoritis, Kastoria, Struma, and Maritza drainages. Asia: Marmara Basin and lower Sakarya in Anatolia, Aral Basin, and Siberia from Ob eastward to Lena drainages (Kottelat and Freyhof, 2007).

The roach is an important economic fish species in freshwater fishing and has an accentuated decreasing trend in both the upstream and downstream of Coştei hydrotechnical work it represents a seventh loss for the local economy.

In situ adapted meandering bypass for the bidirectional and volitional movement of fish over the overflow threshold within the Coştei hydrotechnical node

Conceptual design

For the research area, the key challenge was to design a migration system, so as not to affect the structure and functionality of the overflow dam and its construction elements, namely the left bank of the embankment (dam protected by a wall, over a length of 200 m and protected by grassing over a length of 165 m). Also, the location of a frontal migration system has been avoided, because it is very difficult to achieve access for maintenance.

The solution is to create a fish migration/passage system represented by a meandering by-pass. It is designed for the upstream, downstream, and volitional migration/displacement of the ichthyofauna present in the analyzed area, and also in upstream and downstream sectors.

As the location area of this by-pass, the left bank was selected (a flat plain vast agricultural land), due to the spatial constraints at the level of the right bank (national and local roads and hydrotechnical infrastructure components related to the Coştei hydrotechnical node: i.e. pipeline equipped with drawer valve in the water chamber, and Coştei locality different buildings), as well as the river slope in the area of the analyzed spillway threshold.

Principle of operation

Although from the point of view of space there is the possibility of creating a classic by-pass channel, we opted for a meandering by-pass channel (Fig. 7).

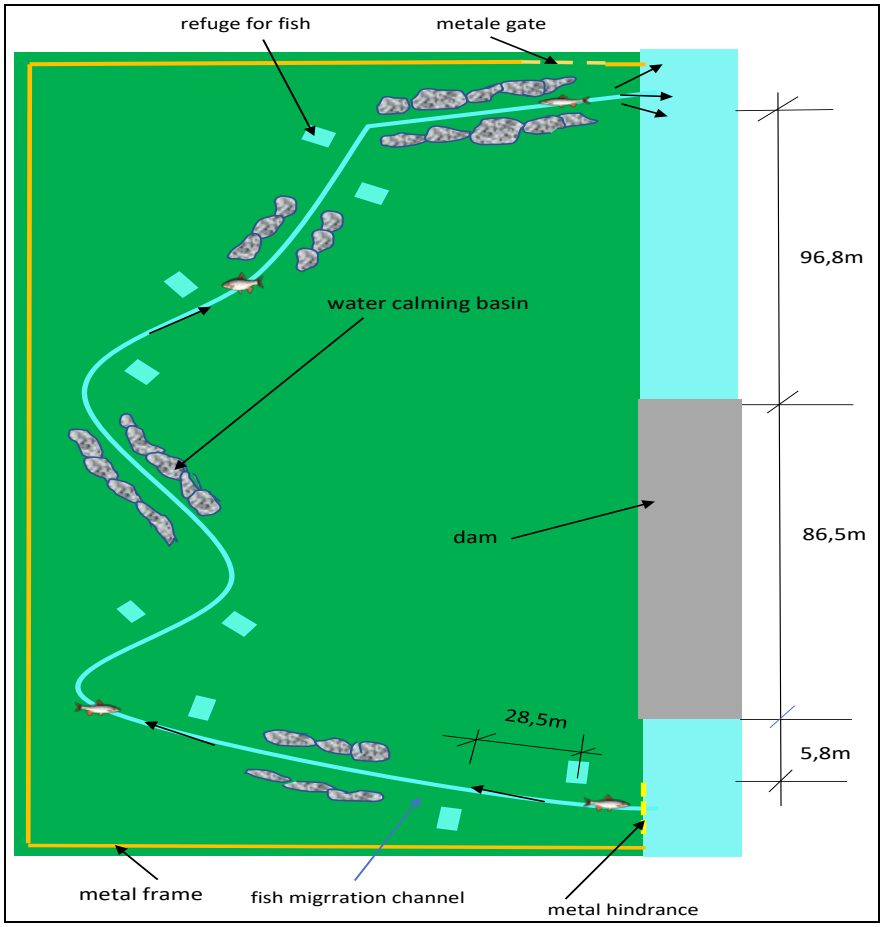

Figure 7: General indicative scheme of the meandering by-pass channel – plan view –.

The inequality in water level between the upstream and the downstream area of the overflow threshold would have led to a by-pass with a too long length. Thus, the aim was to avoid the occurrence of clogging phenomenon, by creating a by-pass that will have the following characteristics: (i) the canal will have a length of approximately 660 m and will be composed of three meanders with fish shelters and rest pools every 28.5 m on both banks; (ii) the water entry in the by-pass channel is located at a distance of 16 m upstream of the overflow ridge, in the area of the left bank; (iii) the water outlet area of the by-pass channel is located at a distance of 96.8 m from the foot of the downstream facing of the overflow threshold (in the axis of the transverse channel). In rest and shelter basins there is always a sufficient water level to allow the fish to re-enter the main stream of the fish migration channel.

Components and dimensions

Description of the meandering by-pass channel

The meandering by-pass canal, proposed for this case study, will have a total length of approximately 660 m. The upstream end of this battlement/crenel (Fig. 8) will be equipped with a dam, fixed on a metal frame (vertical sliding) operated by means of a mechanical reducer and a threaded metal bar (Fig. 9).

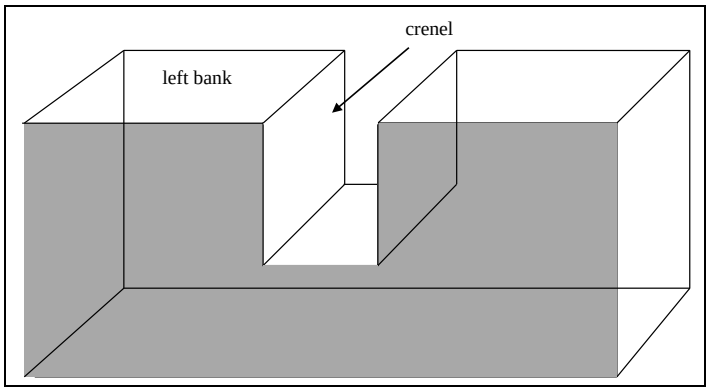

Figure 8: Making the battlement/crenel in the left bank – indicative scheme.

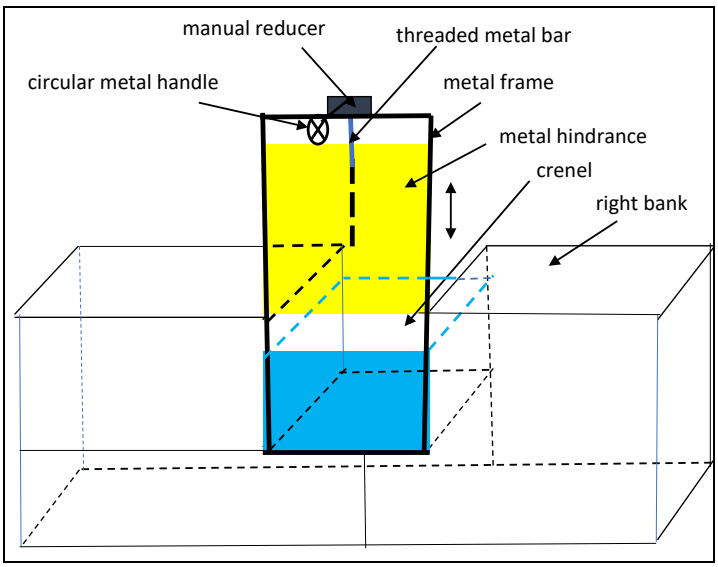

Figure 9: Positioning the dam on the metal frame – indicative scheme.

If for various reasons (e.g. repair work, maintenance, lack of water) it is not desired for the water to flow through the migration system, this dam will be closed.

The downstream end of the meandering by-pass channel will penetrate through the left concrete bank, reaching the watercourse, at a distance of approximately 96.8 m from the foot of the downstream facing (Fig. 10a).

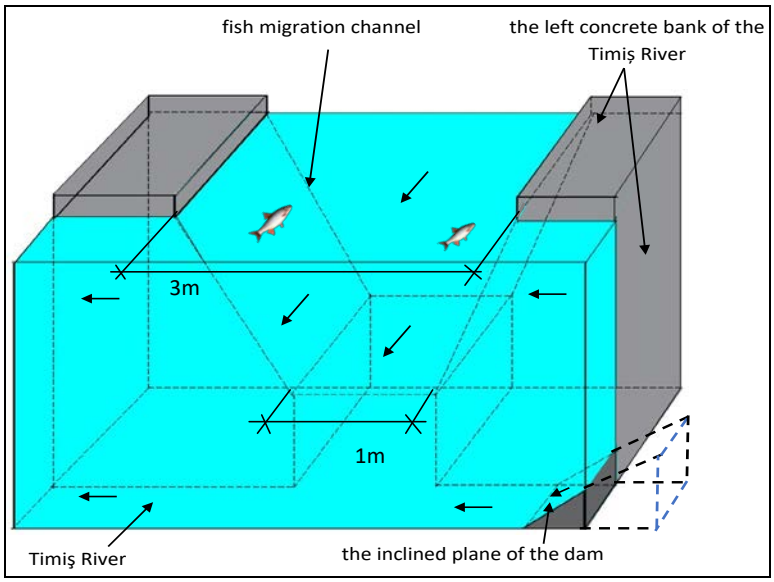

Figure 10a: Positioning the downstream end (entrance) of the meandering by-pass channel – indicative scheme.

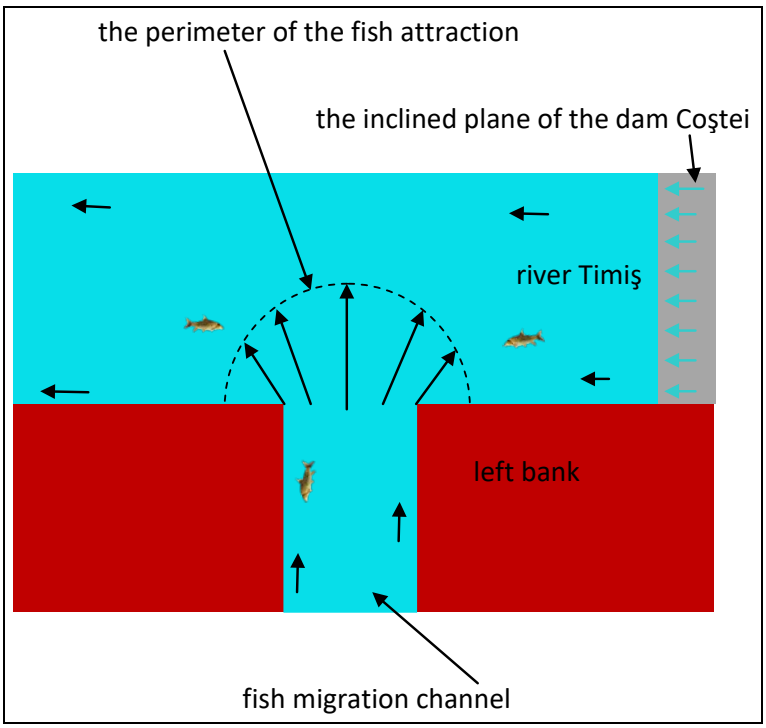

Figure 10b: Positioning the perimeter of the fish attraction – indicative scheme.

In the sense of upstream migration, this will correspond to the area of entry (entrance) of the fish into the meandering channel. Given the amount of water flowing through the by-pass channel, the place of conflict between the fish migration channel and the Timiş River will be detected by fish climbing along the right bank or will be detected by fish moving from the left bank to the right bank. Knowing that migratory fish feel the turbulence in the water from a distance is quite large in our case the radius of the semicircle of attraction (perimeter of attraction) is about three m (Fig. 10.b).

The by-pass channel will have a trapezoidal shape, and will be made of concrete and arranged with stones that also act as sinks (Fig. 11a). On both sides of the concrete channel for the migration of fish, along with it, at distances of about 40 m, will be arranged alternately, concrete steps, necessary for the purpose of interventions (damage, maintenance) (Fig. 11b).

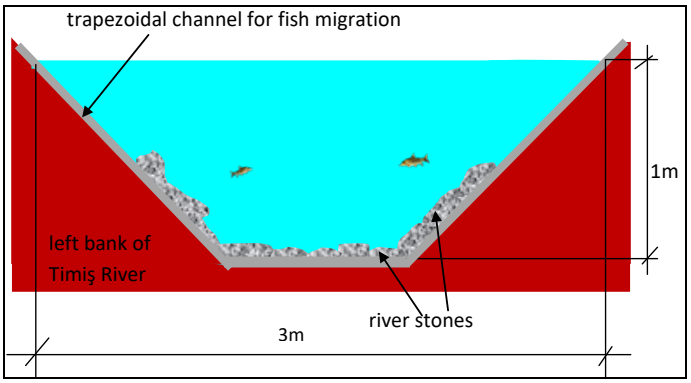

Figure 11a: By-pass channel in cross section – indicative scheme.

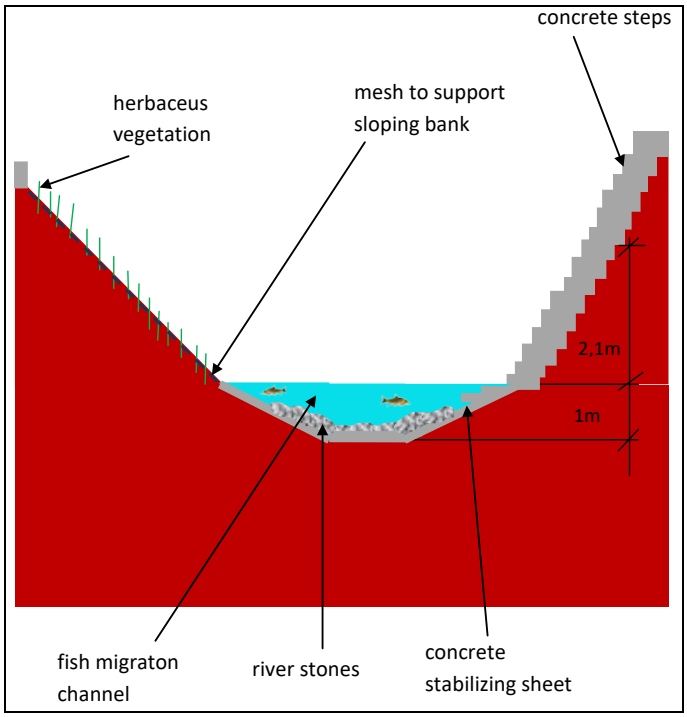

Figure 11b: By-pass channel in cross section – indicative scheme.

Description of resting pools and fish shelters

Taking into account that the ichthyofauna of the study area is represented mainly by cyprinids, the fish shelters will be arranged every 28.5 meters, alternately, inside the banks (by-pass channel) (Fig. 12). They will have a parallelepiped shape, will be paved, and will have the following approximate dimensions: height and depth of ~ one m, and length of ~ three m. Fish shelters, like the entire by-pass, will be flanked by sloping banks (Fig. 11). Their stabilization will be achieved with the help of anti-erosion mattresses and vegetation (herbs).

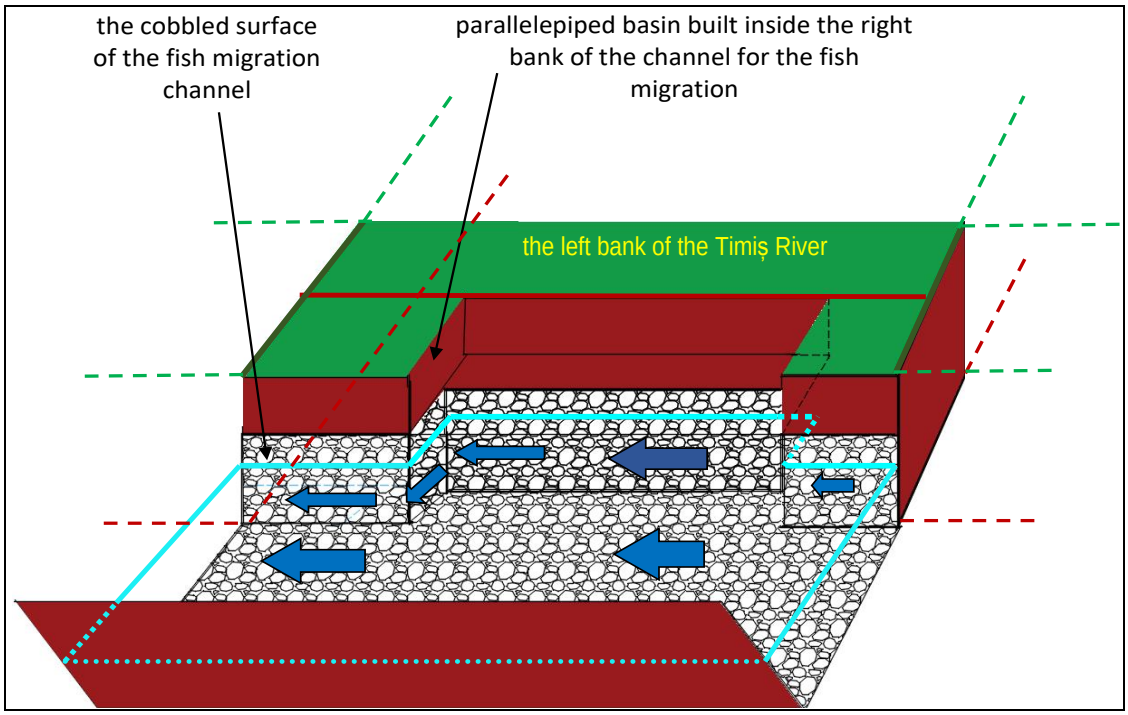

Figure 12: Positioning of fish shelters at the level of the meandering by-pass channel indicative scheme.

Maintenance

Any component can be replaced if damaged. If quality materials are used and the system is built according to the project, it can last a long time.

DISCUSSION

The general agreement of the majority of researchers is that there is a demand for an exhaustive strategy for assessment of diversions and identify direct, indirect, cumulative, and synergic impacts (Wilson et al., 1999; Barbe et al., 2000; Winer and Raphelt et al., 2005; Meselhe et al., 2006).

In the evaluation of impacts of diversions on a broader spatial scale and long-term various changes in the habitats must be considered. Also, various angles of approach which reveal habitat and biocoenoses changes for example habitat changes or succession of riverine vegetation should be studied due to their direct effect on flow within the lotic system, and indirect on aquatic and semi-aquatic animal biota which depends on it, including fish (Curtean-Bănăduc et al., 2014).

The Timiş River in the studied sector but not only (Bănăduc et al., 2013; Curtean-Bănăduc et al., 2018) has been forced to change some of its main ecologic characteristics to fulfil some of the regional human settlements needs, mainly to the needs of a big city (Timişoara) and its metropolitan area, located in a near poorer in water watershed (Bega Basin), consequently in a context of a not ecological technical approach, many natural characteristics of the Timiş lotic system were lost (dynamic balance equilibrium, integrality, homeostazy, resilience, predictability, productivity, etc).

The Coştei diversion system impact was revealed based on the local and regional fish fauna ecological status changes. This approach was based on the fish indicator capacity for aquatic environment changes (López-López and Sedeño-Díaz, 2015; Levin et al., 2019).

The Timiş lotic system ichthyofauna structure was unquestionably altered, due to the drastically hydrogeomorpholgical river changes after the Coştei hydrographical node construction and modernization mainly in terms of water volume, flow and sediment diversion capacity, runoff regime, speed, water quality, etc.

In the situations in which a natural fluctuating of the lotic systems dynamics has been identified as essential to the sustainability of the aquatic systems receiving the diversion flows (Day et al., 1995), the diminishing of the flow induced in Timiş River the more intense rate of sedimentation, with modifications in riverbed morphology, important factors for habitat features changes, and therefore in fish fauna structure modifications. In this respect, it is enough to underline the fact that in the last two centuries were many periods when all the water has deviated to Bega River channel, and nothing remains in the downstream Timiş River (Fig. 5), with exception of few small areas with water from the hyporheic sources (Fig. 6).

This diversion impact is significant, riverbed hydrogeomorphological modifications such as flood routing, sediment load, channel bed aggradations, channel bed degradation, sediment sorting along channel reach, sediment load variation with changing discharge, velocity distribution, secondary circulation, sediment bar formation, shear stress distribution, bedload transport distribution, flow distribution at the bifurcation, and sediment load distribution at bifurcation were followed by ecologic nonlinear/nonproportional to the stimulus responses (Schumman, 1977), highlighted in this study by a skewed ichthyofauna structure, and with decreasing economic benefits related to animal/fish protein accessibility.

Despite of the fact that regarding major hydraulic works it should be established the ecological flow downstream of works and it should be studied their potential impact, and regulating the transfer of water from one reservoir to another so none of the reservoirs should be under or over the optimal volume, no detailed complex hydrogeomorphologic-ecologic analysis was conducted till now about the Coştei diversion node effects before or since its building in 1758, and also not about many of the upstream hydrological works of this basin.

After such a long period of not ecological construction and management of Coştei diversion system, seven main species with high direct economic importance disappeared from the upstream sector, or decrease significantly both in the upstream and downstream sectors, a significant loss for the local natural resources exploitation potential. Also, another nine direct economic important fish species populations which still exists in the upstream lotic sectors are more or less isolated by the lower part of the Timiş River and through it by the adjacent hydrographical nets of Tisza and Danube, having much smaller populations than the river usually supports in the past. Adding to those are more than ten indirect important economic fish species also more or less isolated in the upstream river sectors.

The exposure of this lotic system and its fishes populations to many different coincident or sequential stressors intensifies ecological impacts and vastly complicates restoration and conservation planning, especially where spatially diffuse stressors syndromes span multiple management agencies. The management of freshwater fishes under scenarios of climate change may be the greatest conservation challenge in the studied region where aquatic ecosystems are already exposed to multiple interacting stressors.

Recovery programs should range from individual species to the fauna of entire river basins. Fish conservation should involve the protection and improvement of their habitats and the establishment of refuge habitats. A remarkable effort at basin scale is the rehabilitation of fish communities throughout Timiş River basin, where fishes are subject to severe impacts by many stressors.

In this context, the recovery programs needs an integrated catchment management, restoration of aquatic habitats, dam flow ecological management or removal, provision of environmental flows, restoration of riparian and floodplain processes. Fish populations can be rehabilitated by applying appropriate regulations (e.g. catch and release), no-take zones in critical areas for breeding and recruitment, and even managed relocation and reintroductions. Human activities and stressors that threaten freshwater fishes are likely to become more widespread, intense and damaging unless they are curbed through prevention, improved management, and restoration and adaptation programs.

Even with the most advanced risk evaluation, conservation plans design, fisheries restoration and management tools, enhancements in fish conservation present scientists, managers and citizens with critical challenges and trade-offs, particularly under unusual scenarios of threat under climatic change.

Human pressures on fish must be limited to the maximum degree possible, within constraints of food security, to restore resilience and allow human-assisted adaptations to take effect in the novel and managed environments. Implementation, monitoring and review of fish conservation and management regimes must feed new information back and forth between researchers, managers, and citizens to achieve consensus on what is worth doing, and achievable, in the uncharted waters of the future.

Despite of the revolutionary bioeconomical and ecoeconomical concepts imagined first by the Romanian researcher Nicholas Georgescu-Roegen (IGFA, 1991; Daly, 1999), and developed to be used including in the Romanian ichthyofauna resources context by Grigore Antipa (Antipa, 1909) the not ecological approaches are still a way of acting in the case of Coştei diversion system and not only. Fast ecological approaches and technical solutions are needed to recover at least a part of the economical and ecological losses in the studied region ichthyofauna resources.

Ichthyocenoses are recognized as key components for lotic ecosystems. The structure (qualitative and quantitative) and dynamics (spatial and temporal) of fish communities are conditioned by the diversity and quality of characteristic habitats (natural or man-made), the quantity and quality of available trophic resources, interaction with other communities, etc. (Lasne et al., 2007; Kennen et al., 2008; Hermoso et al., 2009; Infante et al., 2009). The skewed structure of the ichthyofauna negatively influenced by the Coştei diversion system construction and management over more than two centuries, in a general and complex human influenced river system, obviously reflect drastic long-term ecosystem changes, including on fish fauna.

If no specific adapted measures will urgently be taken, like those proposed in this work, in the global changes actual situation (Oprean, 2012) the climate ecological trend will continue possibly in this revealed direction new unexpected ecological nonlinear/nonproportional effects.

CONCLUSIONS

The Timiş River watershed former and current water resource inapropriate management way is inaccurate and potentially harmful for the human enterprise. The proposed technical solution implementation can be a successful intervention to restore Timiş River ecosystems in a long term sustainable way, prevent local extinctions of some fish species and encourage more local and regional economic activities, and support from a sustainable perspective the fish species local and regional populations ecological status.

The upstream and downstream sectors of Coştei hydrographical node, are characterized by similar physiogeographic characteristics, which include location, catchment size, geological structure, drainage density, land denivelations, and land use, but the studied diversion system in the context of more other significant human impacts in the basin, induced long term habitat changes and drastic fish fauna changes, especially in the upstream river sector.

The Coştei diversion system ecological problems are important not only for the heavily human impacted Timiş Basin fish fauna, but in a larger frame of ecological human induced fragmentation for the Tisza Basin fish fauna too.

The specific on-site adapted technical solution proposes the realization of a meandering bypass for the bidirectional and volitional movement of fish over the overflow threshold within the hydrotechnical node, helping in improving the fish populations' connectivity and ecological status for the entire Timiş and Tisza basins.

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A good thought to my childhood, teenage and not only best friend Adam Helmut Johann with which we discovered together the extraordinary "universe" of Timiş River.

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Annex 1/Table 1: Historic local data about fish obtained from local top fisherman about the species of interest trend; decreasing abundance 🡻, increasing abundance 🡹.

Fish species Interviewed top local fisherman	*Silurus glanis*	*Cyprinus carpio*	*Lota lota*	*Sander lucioperca*	*Tinca tinca*	*Abramis brama*	*Rutilus rutilus*
Agăsân Andraş Covered period 1905-1985	🡻	🡻	🡻	🡻	🡻	🡻	🡻
Adam Josef and Adam Helmut Johann Covered period 1945-2020	🡻	🡻	🡻	🡻	🡻	🡻	🡻

Annex 2/Table 2a: Local data about fish species of interest trend obtained from 10 fisherman/one day/one year; present species in the dedicated for fish species of interest fisherman capture + (minimum 10 hours of fishing per day), absent species –.

10 local on site fisherman captures analyse	Year	*Silurus glanis*	*Cyprinus carpio*	*Lota lota*	*Sander lucioperca*	*Tinca tinca*	*Abramis brama*	*Rutilus rutilus*
	1996							
Fishermen 1		+	–	–	–	–	–	–
Fishermen 2		–	+	+	–	–	+	–
Fishermen 3		–	–	–	–	–	–	–
Fishermen 4		+	–	+	–	+	–	+
Fishermen 5		–	–	–	–	–	+	–
Fishermen 6		–	–	+	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		–	–	+	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		+	–	–	–	–	–	–
	1999							
Fishermen 1		–	+	–	–	–	–	–
Fishermen 2		–	+	–	–	–	–	+
Fishermen 3		–	+	–	–	–	–	–
Fishermen 4		–	–	–	+	–	–	–
Fishermen 5		–	–	+	–	+	+	–
Fishermen 6		+	–	+	–	+	+	–
Fishermen 7		–	–	–	–	+	–	–
Fishermen 8		–	–	–	–	+	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–

Annex 2/Table 2b: Local data about fish species of interest trend obtained from 10 fisherman/one day/one year; present species in the dedicated for fish species of interest fisherman capture + (minimum 10 hours of fishing per day), absent species –.

	2002							
Fishermen 1		–	–	–	–	–	–	–
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		+	–	–	–	–	–	–
Fishermen 4		–	–	–	–	–	–	–
Fishermen 5		–	–	+	–	–	–	–
Fishermen 6		+	–	–	–	–	–	–
Fishermen 7		–	–	–	–	–	–	+
Fishermen 8		–	–	–	–	–	–	+
Fishermen 9		+	–	–	+	–	–	–
Fishermen 10		+	–	–	–	–	–	–
	2005							
Fishermen 1		–	–	–	–	–	–	–
Fishermen 2		–	–	+	–	–	–	–
Fishermen 3		–	–	+	–	–	–	–
Fishermen 4		–	+	–	–	+	+	–
Fishermen 5		+	+	–	–	–	–	–
Fishermen 6		–	–	–	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		–	–	–	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–

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Annex 2/Table 2c: Local data about fish species of interest trend obtained from 10 fisherman/one day/one year; present species in the dedicated for fish species of interest fisherman capture + (minimum 10 hours of fishing per day), absent species –.

	2008			–			–	–
Fishermen 1		–	–	–	–	–	+	–
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		–	–	–	–	–	–	–
Fishermen 4		–	–	–	–	–	–	–
Fishermen 5		–	–	–	–	–	–	–
Fishermen 6		–	–	–	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		–	–	–	–	–	–	+
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		+	–	–	–	–	–	–
	2011							
Fishermen 1		–	–	–	–	–	–	–
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		–	–	+	–	–	–	–
Fishermen 4		+	–	–	–	–	–	–
Fishermen 5		–	–	–	–	–	–	–
Fishermen 6		–	–	–	–	–	–	–
Fishermen 7		+	–	–	–	–	–	–
Fishermen 8		–	–	–	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–

Annex 2/Table 2d: Local data about fish species of interest trend obtained from 10 fisherman/one day/one year; present species in the dedicated for fish species of interest fisherman capture + (minimum 10 hours of fishing per day), absent species –.

	2014							
Fishermen 1		–	+	–	–	–	–	+
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		–	–	–	–	+	–	–
Fishermen 4		–	–	–	–	–	–	–
Fishermen 5		–	–	–	–	–	–	–
Fishermen 6		–	–	+	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		–	–	–	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–
	2017							
Fishermen 1		–	–	–	–	–	–	–
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		–	–	–	–	–	–	–
Fishermen 4		–	–	–	–	–	–	–
Fishermen 5		–	–	–	–	–	–	–
Fishermen 6		–	–	–	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		+	–	–	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–

Annex 2/Table 2e: Local data about fish species of interest trend obtained from 10 fisherman/one day/one year; present species in the dedicated for fish species of interest fisherman capture + (minimum 10 hours of fishing per day), absent species –.

	2020							
Fishermen 1		–	–	–	–	–	–	–
Fishermen 2		–	–	–	–	–	–	–
Fishermen 3		–	–	–	–	–	–	–
Fishermen 4		–	–	–	–	–	–	–
Fishermen 5		–	–	–	–	–	–	–
Fishermen 6		–	–	–	–	–	–	–
Fishermen 7		–	–	–	–	–	–	–
Fishermen 8		–	–	–	–	–	–	–
Fishermen 9		–	–	–	–	–	–	–
Fishermen 10		–	–	–	–	–	–	–