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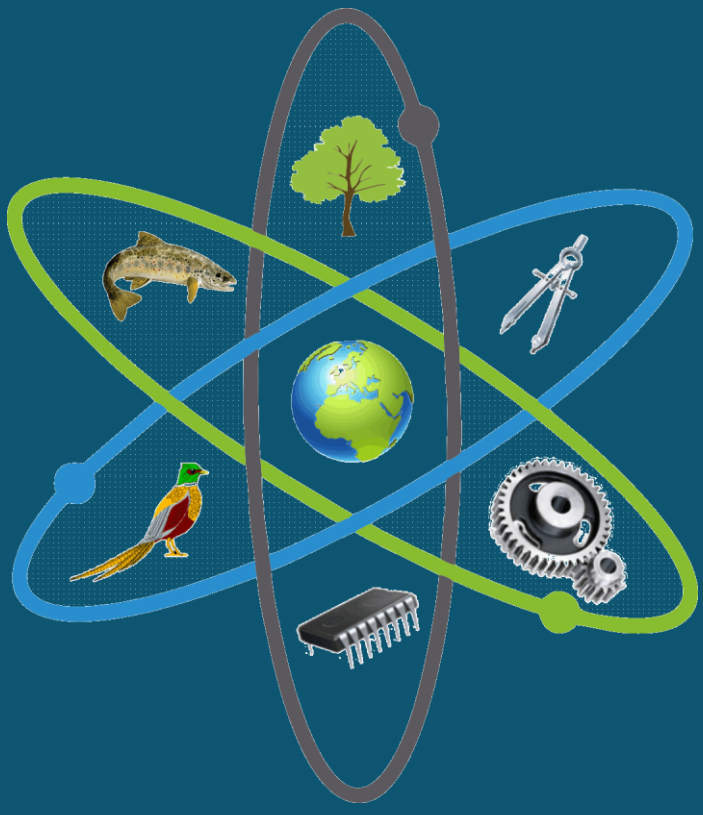

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## Contents

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# Evaluation of Antimicrobial Activities of *Salvia verbenaca*

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## Abstract

The antimicrobial activity of several extracts and fractions of *Salvia verbenaca* L. (Lamiaceae) was investigated by disc diffusion and broth microdilution methods against *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *Micrococcus flavus* ATCC 14452, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 19115, *Mycobacterium smegmatis* CCM 2067, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Cryptococcus neoformans* ATCC 90112, *Kluyveromyces fragilis* NRRL 2415 and *Rhodotorula rubra* DSM 70403. The methanol extract, butanol and chloroform fractions have shown potential antimicrobial effects against some bacteria and the yeast cultures tested, with grown inhibition area diameters in the range 10.8 – 22.4 mm, and MIC values between 0.03 and 0.34 µL/mL. The results of the study support the use of the plant in traditional medicine.

**Keywords:**

*Salvia verbenaca, antimicrobial activity, plant extract*

**Article history:**

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## Introduction

The genus *Salvia* L. is one of the most species-rich genera of flowering plants, with about 1000 species currently accepted (Gonzales-Gallegos et al., 2020). Besides, *Salvia* L. is widely distributed

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in subtropical and temperate regions. Turkey has the second highest number of *Salvia* species in the world, after Mexico, with about 100 species, 53 of which are endemic (Celep et al., 2020).

*Salvia* species are commonly used in Anatolia for colds, stomach aches, and sore throats (Tabanca et al., 2017), to treat inflammatory skin diseases, to stop bleeding, or as an antiseptic for wounds (Suntar et al., 2011).

The secondary metabolites, namely essential oils and plant extract of some *Salvia* species are known to possess antioxidant, antimicrobial, antifungal, and aromatic properties (Gali-Muhtasib et al., 2000; Doğan et al., 2007). Therefore, they are intensely screened and applied in the fields of pharmacology, medicine, and food preservation (Cowan, 1999).

*Salvia verbenaca* L. (Lamiaceae) is a perennial herbaceous plant, endemic to the Mediterranean region and the Canary Islands and has expanded into Europe and Asia (Codd Lesli and Leistner, 1985). The species is cultivated in several countries mainly to produce dried leaves as raw material in herbal medicine (Baser, 2000). *S. verbenaca* is used as a bactericide against respiratory diseases, as eyedrops (Canzoneri et al., 2011), and in healing wounds and ulcers.

As a result of the interviews, it was determined that *S. verbenaca* was used by the local people for wound treatment and eye disinfection during routine trips. Hence, here the purpose was to determine the antimicrobial effects of the ethanolic leaf extract of this plant collected from Turkey, which is used by the local people to cure some illnesses.

## Materials and Methods

### *The Plant Material*

The plant samples were collected during to flowering stage in June 2020 at an altitude of 765 m, Samandere village around, Düzce, Turkey. A voucher specimen of the plant was deposited in the Department of Medical Biology of Duzce University in the author's collection (Voucher number; GD.109-8).

### *Preparation of Extracts*

Plant samples were dried in an oven at 40 °C and powdered. Methanol extracts were obtained by maceration of the plant material with methanol for 3 days at room temperature, the procedure was done three times. The extracts were filtered and dried under reduced pressure at a temperature below 45°C. Then the methanol extracts were separated between chloroform and water ($CHCl_3$, $H_2O$). Finally, the aqueous fraction was again subjected to separation between n-butanol and water (n-BuOH, $H_2O$). The yields obtained for each extract and fraction as percentages of the initial dry material were *S. verbenaca* 24.20% for MeOH ($CHCl_3$ 9.82%, BuOH 22.10%, aqueous 31.46%).

Infusions were prepared with 100 g crude powder and 1000 mL water, and the volume was adjusted to a concentration of 1 g/mL under reduced pressure at 40 °C.

***Preparation of Samples***

The different extracts and fractions were diluted in dimethylsulfoxide (DMSO). The corresponding concentrations are expressed as mg extract or fraction per mL solvent, except for infusions, whose concentrations are expressed as mg initial dry material per mL. For each experiment, a disc containing only (DMSO) was used as a control.

***Bioassays***

The antimicrobial tests employed the disc diffusion method (Bauer et al., 1966), and the minimum inhibitory concentration (MIC) was determined by microdilution according to the microplate method (Rabanal et al., 2002; Jones et al., 1987).

The microorganisms to be tested were inoculated into Brain Heart Infusion Agar (Oxoid) for the bacteria, and Saboraud Dextrose Agar (Oxoid) for the yeasts. After 24h incubation at 35°C and 28°C, respectively, three or four colonies isolated from the media were incubated into 4 mL of Brain Heart Infusion Broth (Oxoid) for the bacteria, and Saboraud Dextrose Broth (Oxoid) for the yeasts, and incubated for 2h at 35°C and 28°C, respectively. The cultures were adjusted with sterile saline solution to turbidity comparable to that of McFarland (0.5) standard. Petri dishes containing Mueller Hinton Agar (Oxoid) or Bacto Yeast Morphology Agar (Difco) were impregnated with these microbial suspensions for the bacteria and yeasts, respectively (Bauer et al., 1966; Rabanal et al., 2002).

MIC was estimated by the broth microdilution method in M24A microplates against the most sensitive microorganisms, using liquid media containing decreasing amounts of the test materials. From the initial solution extract of 375 mg/mL, double dilutions in the culture medium (Mueller Hinton Broth for bacteria, Sabouraud Dextrose Broth for yeasts) were prepared and tested at concentrations ranging from 37.5 to 0.03 mg/mL. After mixing, 5 µL cell suspension (105 cells per µL) was added and mixed vigorously again. The temperature is for 24h in a humid atmosphere. Afterward, the microplates were centrifuged and examined for growth. All data represented at least three replicated experiments per microorganism.

***Microorganisms***

*Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *M. flavus* ATCC 14452, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 19115, *Mycobacterium smegmatis* CCM 2067, *Bacillus cereus* ATCC 7064, *B. subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Cryptococcus neoformans* ATCC 90112, *Kluyveromyces fragilis* NRRL 2415 and

*Rhodotorula rubra* DSM 70403 were collections maintained in the Laboratory of Medical Biology, Faculty of Medicine at Düzce University.

## Results

The antimicrobial activity of the methanol extracts of *S. verbenaca* and standard comparison antibiotics assessed by the disc diffusion method are given in Table 1. No significant effects were found against *Micrococcus luteus*, *M. flavus*, *Listeria monocytogenes*, and the acid-fast bacterium *Mycobacterium smegmatis*. The methanolic extracts of the plant showed potential antimicrobial activity against the test microorganisms, with inhibition zones at 10.8 to 22.4 mm.

Table 1. Antimicrobial activity of *S. verbenaca* methanol extracts as found by the disc diffusion method

| Tested microorganisms | *S. verbenaca* MeOH extract (Diameter of inhibition zones (mm)) | | | Standard antibiotics | |
|---|---|---|---|---|---|
| | 1.25 mg/mL | 2.50 mg/mL | 3.75 mg/mL | 1 (0.03 mg/mL) | 2 (0.10 mg/mL) |
| ***Escherichia coli*** | 14.2 | 15.6 | 15.8 | 24.6 | NT |
| ***Staphylococcus aureus*** | 18.2 | 20.4 | 22.4 | 30.2 | NT |
| ***Klebsiella pneumoniae*** | 12.6 | 13.8 | 14.2 | 22.8 | NT |
| ***Micrococcus luteus*** | - | - | - | 34.2 | NT |
| ***Micrococcus flavus*** | - | - | - | 30.6 | NT |
| ***Proteus vulgaris*** | 14.6 | 17.8 | 20.4 | 24.4 | NT |
| ***Pseudomonas aeruginosa*** | 12.2 | 12.6 | 13.0 | 14.6 | NT |
| ***Listeria monocytogenes*** | - | - | - | 22.2 | NT |
| ***Mycobacterium smegmatis*** | - | - | - | 20.6 | NT |
| ***Bacillus cereus*** | 14.2 | 14.2 | 15.6 | 23.4 | NT |
| ***Bacillus subtilis*** | 13.2 | 14.4 | 16.0 | 26.8 | NT |
| ***Candida albicans*** | 14.0 | 16.2 | 18.2 | NT | 14.4 |
| ***Cryptococcus neoformans*** | 11.2 | 12.8 | 14.6 | NT | 17.8 |
| ***Kluyveromyces fragilis*** | 10.8 | 11.2 | 12.4 | NT | 16.2 |
| ***Rhodotorula rubra*** | 14.2 | 15.6 | 16.2 | NT | 14.6 |

(-): no inhibition zones; NT: Not tested; 1: Chloramphenicol; 2: Amphotericin B

The antimicrobial activity of the aqueous, butanol, and chloroform fractions obtained from the methanol extracts of the plant are presented in Table 2. Antimicrobial activity was not observed in the aqueous fraction against all the tested microorganisms. The diameter of the growth inhibition

area ranged from 10.2 to 17.2 for the BuOH fraction and 10.2 to 15.4 for the $CHCl_3$ fraction. The extracts of the plant showed the highest activity against *Staphylococcus aureus* and *Proteus vulgaris* (inhibition values close to that of Chloramphenicol) and *Candida albicans* and *Rhodotorula rubra* (inhibition values close to that of Amphotericin B). The inhibition zone diameters around the control disc (containing only DMSO) were 0-0.5 mm.

Table 2. Antimicrobial activity of *S. verbenaca* fractions (aqueous, BuOH, and $CHCl_3$) as found by the disc diffusion method

| **Tested microorganisms/ The plant fractions** | **Aqueous fraction** | | | **BuOH fraction** | | | **$CHCl_3$ fraction** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1.25 mg/mL** | **2.50 mg/mL** | **3.75 mg/mL** | **1.25 mg/mL** | **2.50 mg/mL** | **3.75 mg/mL** | **1.25 mg/mL** | **2.50 mg/mL** | **3.75 mg/mL** |
| ***Escherichia coli*** | - | - | - | 12.2 | 13.4 | 14.6 | 11.2 | 12.0 | 12.0 |
| ***Staphylococcus aureus*** | - | - | - | 14.6 | 15.4 | 17.2 | 13.2 | 14.6 | 15.0 |
| ***Klebsiella pneumoniae*** | - | - | - | 11.4 | 12.2 | 12.6 | 14.0 | 13.2 | 14.2 |
| ***Proteus vulgaris*** | - | - | - | 13.2 | 14.6 | 15.2 | 13.2 | 15.2 | 14.6 |
| ***Pseudomonas aeruginosa*** | - | - | - | 11.4 | 12.0 | 12.4 | 10.6 | 11.0 | 11.2 |
| ***Bacillus cereus*** | - | - | - | 12.6 | 14.2 | 15.0 | 11.2 | 12.0 | 13.2 |
| ***Bacillus subtilis*** | - | - | - | 12.8 | 14.6 | 16.2 | 12.2 | 13.0 | 12.4 |
| ***Candida albicans*** | - | - | - | 14.0 | 15.2 | 17.2 | 14.2 | 15.0 | 15.4 |
| ***Cryptococcus neoformans*** | - | - | - | 10.2 | 11.0 | 11.0 | 10.2 | 11.0 | 11.6 |
| ***Kluyveromyces fragilis*** | - | - | - | 10.4 | 10.6 | 11.0 | 10.6 | 11.2 | 12.6 |
| ***Rhodotorula rubra*** | - | - | - | 13.6 | 14.2 | 15.4 | 12.4 | 13.2 | 14.0 |

(-): no inhibition zones

Table 3 summarized the MIC values of the active extracts and fractions. These values ranged from 0.03 to 0.34 mg/mL. The highest antibacterial activity was observed in the $CHCl_3$ fraction with particularly low MIC values against *Bacillus subtilis* and *Staphylococcus aureus* (0.03 mg/mL) following against *Bacillus subtilis* (0.05 mg/mL). *Rhodotorula rubra* is susceptible to $CHCl_3$ fraction among the yeasts at MIC values of 0.05 mg/mL. In general, the extracts and fractions obtained from *S. verbenaca* had a potential antimicrobial effect on bacteria especially *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus* as well as the yeast culture *R. rubra*.

Table 3. Minimum inhibitory concentration as found by the microdilution method

| Tested microorganisms | MIC (mg/mL) | | | | |
|---|---|---|---|---|---|
| | Extract/Fractions | | | Standard antibiotics | |
| | MeOH extract | BuOH fraction | $CHCl_3$ fraction | 1 | 2 |
| ***Escherichia coli*** | 0.22 | 0.22 | 0.18 | 0.002 | NT |
| ***Staphylococcus aureus*** | 0.09 | 0.09 | 0.03 | 0.002 | NT |
| ***Klebsiella pneumoniae*** | 0.29 | 0.34 | 0.22 | 0.002 | NT |
| ***Proteus vulgaris*** | 0.11 | 0.09 | 0.05 | 0.006 | NT |
| ***Pseudomonas aeruginosa*** | 0.29 | 0.34 | 0.22 | 0.008 | NT |
| ***Bacillus cereus*** | 0.09 | 0.09 | 0.05 | 0.0005 | NT |
| ***Bacillus subtilis*** | 0.09 | 0.09 | 0.03 | 0.0005 | NT |
| ***Candida albicans*** | 0.09 | 0.09 | 0.05 | NT | 0.008 |
| ***Cryptococcus neoformans*** | 0.29 | 0.29 | 0.29 | NT | 0.002 |
| ***Kluyveromyces fragilis*** | 0.29 | 0.29 | 0.22 | NT | 0.006 |
| ***Rhodotorula rubra*** | 0.11 | 0.09 | 0.05 | NT | 0.006 |

NT: Not tested; 1: Chloramphenicol; 2: Amphotericin B

**Discussion**

In this study, the methanol extract, butanol and chloroform fractions obtained from *Salvia verbenaca* L. (Lamiaceae) was investigated by disc diffusion and broth microdilution methods. The extracts have shown potential antimicrobial effects against some bacteria and the yeast cultures tested, with grown inhibition area diameters in the range 10.8 – 22.4 mm, and MIC values between 0.03 and 0.34 µL/mL. When similar previous studies on the antibacterial activity of *S. verbenaca* were examined, Al-Howiriny (2002) investigated the MIC values of the oil obtained from *S. verbenaca* were 2.0 mg/mL against *B. subtilis* and *S. aureus*, and 3.0 mg/mL against *M. smegmatis* but *E. coli* and *P. aeruginosa* were resistant to the oil. Salah et al. (2006) studied the antibacterial activity in the methanolic extract of *S. verbenaca* leaves against *P. aeruginosa* and reported a MIC value greater than 1000 µg/mL. Kamatou et al. (2007) determined the antibacterial effects of methanolic extract of aerial parts of *S. verbenaca* against *E. coli*, *K. pneumoniae*, *B. cereus*, and *S. aureus*, with MIC values of 8, 2, 2, and 3 mg/mL, respectively. Sarac & Ugur (2007) reported the growth inhibitory effect of ethanol extract of *S. verbenaca* against various pathogenic bacteria with

inhibition zones ranging between 9-11 mm. In another study, ethyl acetate extract of *S. verbenaca* from aerial part growth in Algeria was investigated against eight microorganisms. The authors studied the effect of two different concentrations (100 mg/mL and 200 mg/mL) and indicated a proportional effect of *S. verbenaca* ethyl acetate extract concentration. At 200 mg/mL, the inhibitory zone varied between 13 and 16 mm, with a large inhibitory zone was reported against *S. aureus* (16 mm) (Belkhiri et al., 2017). Kabouche and Kabouche (2008) investigated the antibacterial activity of root acetone extract at 128 mg/mL against several strains (Kabouche and Kabouche, 2008). The results indicated that *B. subtilis* (28 mm, MIC=4 µg/mL). *S. aureus* (26 mm, MIC=26 µg/mL) and *Streptococcus haemolyticus* (22 mm, MIC=6 µg/mL) were extremely sensitive to the concentration of 128 mg/mL, while a weak antibacterial effect was reported for *E. coli*, *K. pneumoniae*, *P. mirabilis* and *S. haemolyticus*. In our study, extract and fractions showed predominantly activity against *S. aureus*, *B. cereus* and *B. subtilis* as Gram positive bacteria. Notably, *P. vulgaris* as Gram negative bacterium is susceptible to the extract and fractions. The other Gram-negative bacteria have shown smaller diameters of inhibition zones. The results obtained from this section are similar to the previously the mentioned literature results. Besides, the structure of the cellular wall of Gram-positive bacteria appeared to be more sensitive to the plant extracts compared to the Gram-negative bacteria cellular wall, which is also composed of several layers of peptidoglycan but additionally surrounded by a membrane containing fatty substances and polysaccharides, giving it less permeable characteristics compounds (Kozlowka et al., 2022).

There are limited studies on *S. verbenaca* examining its antifungal activities (Al-Howiriny, 2002; Salah et al., 2006). Al-Howiriny (2002) studied the antifungal activity of essential oil extracted from aerial parts of *S. verbenaca* against Candida albicans and observed a MIC value of 2.0 mg/mL. Salah et al. (2006) investigated the antifungal effect of *S. verbenaca* leaves against *C. albicans* and *Cryptococcus neoformans* and determined MIC values greater than 1000 µg/mL for both strains. In our studies, extract and fractions showed predominantly antifungal effects against *C. albicans* and *R. rubra*. A weaker activity against the other the yeast cultures as *C. neoformans* and *K. fragilis* have determined. Our findings in this section are partially similar to the results of the literature data the mentioned above. According to the findings of Sas-Piotrowska and Piotrowski (2003), the biological activity of plant extracts depends on several factors, and first of all on content of specific chemical compounds and on their ability to diffuse. Besides that, some those compounds may stimulate a pathogen development and increase a degree of contamination and the others can act as inhibition factors differences between action of brew, macerate, decoction and oils probably resulting from possible losses caused by evaporation of the solvent during preparation and the difference in the solubility of the extracted.

Studies on the bioactivity of *S. verbenaca* have shown that it is an important antimicrobial agent depending on different factors such as the extract used, the localization of the plant, the collection time, the part used, the extraction methods, the experiments used and the bioactive

compounds in the plant (Bouyahya et al., 2018). However, the mechanism by which extracts and essential oils are activated is not fully understood. Indeed, different investigations reported that bioactive molecules such as flavonoids belonging to the flavanones subclass showed important antibacterial activity by decreasing biofilm formation and decreasing fatty acid secretion (Song et al., 2020). Moreover, modifying cell morphology and gene expression, increasing cell permeability, and inhibiting the quorum-sensing system are also mechanisms of pathways by which molecules exert their effects on bacteria (Bouyahya et al., 2019). Furthermore, the synergistic effects of the major and the minor phenolic compounds should be taken into consideration.

In conclusion, this study may suggest that various extracts of *S. verbenaca* possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for the therapy of infectious diseases in humans, especially against *S. aureus*, *Bacillus* species, *C. albicans* and *R. rubra*.

**Conflict of Interest**

The authors declare that they have no competing interests.

**Author Contributions**

All authors' contributions are equal for the preparation of research in the manuscript.

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# Existence of *Belone svetovidovi* Collette & Parin, 1970 in the Marmara Sea and Black Sea Coasts of Türkiye

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## Abstract

The first morphologic and genetic documentation of the short-beaked garfish *Belone svetovidovi* Collette & Parin, 1970 from the coast of the Marmara Sea (Yalova) and Black Sea (Akcakoca) is reported in the present study. The morphological characters and genetic (mtDNA COI) analyses confirmed the existence of this species both in the Marmara and Black Seas. *B. svetovidovi* is genetically distinct from the anther species of this genus *Belone belone*. All morphologic measurements, counts, and colour descriptions of *B. svetovidovi* agree with its previous descriptions.

**Keywords:**

*Belonidae, short-beaked garfish, first record, existence, Marmara Sea, Black Sea*

**Article history:**

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## Introduction

The short-beaked garfish *Belone svetovidovi* Collette & Parin, 1970 belongs to the Belone genus and represents the Belonidae family. This family is comprised of three species *(Belone belone*,

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*Belone svetovidovi* and *Tylosurus acus*) on the coast of Türkiye (Collette & Parin 1986; Karataş et al., 2021).

The short-beaked garfish *B. svetovidovi* is a benthic fish species and prefers tropical and temperate waters, and found between depths of 1 to 20 m (Froese and Pauly, 2023) in the Eastern Atlantic and Mediterranean, where its range extends from southern Ireland, Spain, Portugal, Israel and Turkey (Akşıray, 1987; Golani, 2006; Froese and Pauly, 2022). In Turkish marine waters, *B. svetovidovi* was first time recorded in the Aegean Sea (Meriç and Altun, 1999), and then this species was reported from the Turkish coast of the Eastern Mediterranean (Iskenderun Bay) by Dalyan & Eryılmaz (2005), and taken place in the Turkish checklists (Fricke et al., 2007; Karataş et al., 2021).

In the present study, the existence of the short-beaked garfish *B. svetovidovi* from the Marmara and Black Sea coasts of Turkiye is given for the first time with both morphologic and genetic evidence. Therefore, the finding of *B. svetovidovi* herein presented constitutes the first well-documented record with taxonomic and molecular identification of the species from the Turkish coasts of the Marmara Sea and Black Sea. It also contributes significantly to the inclusion of this species in the Turkish marine fish checklist with evidence of the presence of this species from the Marmara Sea and Black Sea.

## Materials and Methods

### *Sampling and Morphological Analysis*

The five short-beaked garfish *Belone svetovidovi* specimens were collected as gill net at the Yalova coast on November 11, 2022, in the Marmara Sea (40.682854 N, 29.268145 E), depth 10 m), and five specimens were captured at the coast of Akcakoca in the Black Sea (41.122554 N, 31.122507 E) on September 2022 (Figure 1). All captured specimens were immediately frozen and transported to the laboratory for detailed morphological and genetic analysis. Each body length (±0.1 mm) and total body weight (W) (±0.01 g) were measured. Sex and gonad maturity stages were assessed macroscopically. All specimens of *B. svetovidovi* (Figure 2) were carefully examined and identified using field guides and ichthyological fauna (Collette and Parin, 1986; Golani et al., 2006).

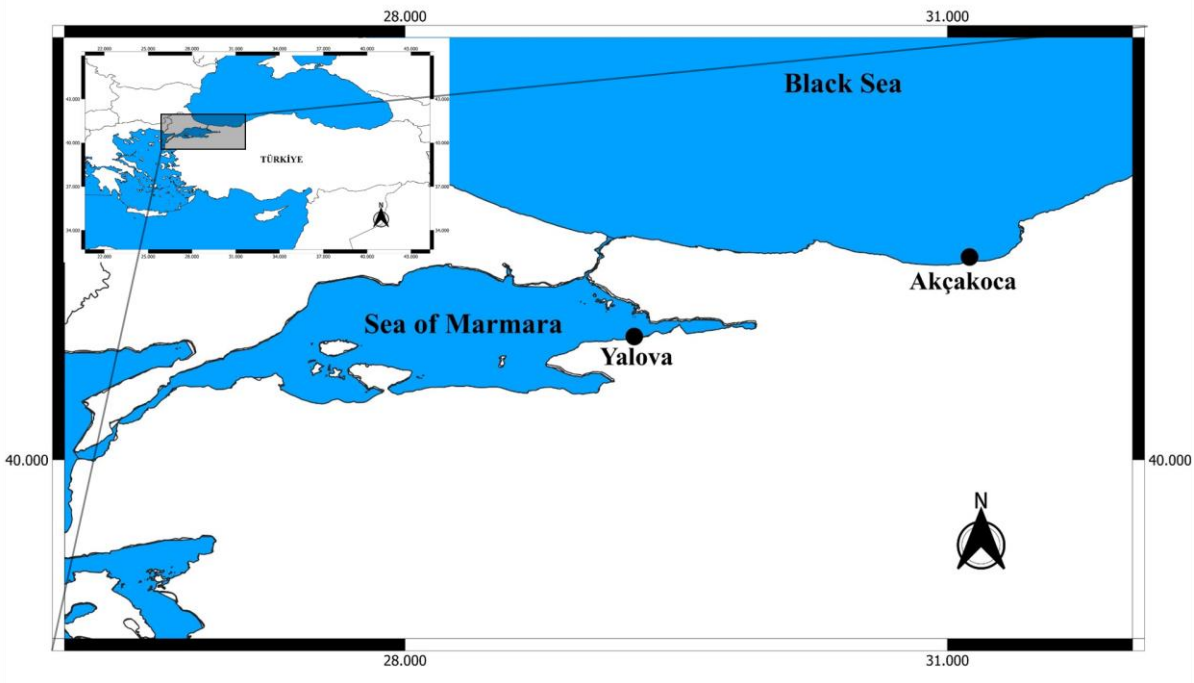


Figure 1. Sampling locations of *Belone svetovidovi.*


Figure 2. *Belone stevetovidovi* (a: General view, b: Beak and head structure, c: The back, anus and caudal fin).

***Molecular Analysis***

Total genomic DNA from five specimens of each sea was extracted from the muscle and fin samples using the DNeasy Blood and Tissue Kit (Qiagen, USA). The manufacturer's protocols were used during all steps. The mtDNA COI gene region was amplified through PCR with universal primers (Ward et al., 2005). Fish_F: 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-'3' -Fish_R: 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-'3'.

The PCRs were conducted in a 50 µl total volume with 0.4 uM of each primer, 0.2 mM of dNTP and 1.25U of Taq DNA polymerase in a PCR buffer that included 20 mM of Tris–HCl (pH 8.0), 1.5 mM of MgCl2, 15 mM of KCl and 1-2 µl template DNA. The denaturation step was at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s for 30 cycles and followed by a final extension for 7 min at 72 °C. The PCR products were visualized using electrophoresis on 1.5 % agarose gel. DNA sequencing was attempted to determine the order of the nucleotides of the mtDNA COI gene region. The chain termination method by Sanger et al. (1977) was applied with Bigdye Cycle Sequencing Kit V3.1 and ABI 3130 XL genetic analyzer. The initial alignments of partial COI sequences were performed with the Clustal W program (Thompson et al., 1994), and the final alignment was completed manually with BioEdit (Hall, 1999). The best-fit substitution model (HKY + G) was provided by the MEGAX software (Kumar et al., 2018). After sequence alignment, MEGA X was used to determine the genetic diversity and sequence divergences and to construct the phylogenetic tree (Kumar et al., 2018). Sequences of *Belone belone* and *Sphyraena sphyraena* were obtained from Genbank and Bold System.

**Results**

*Belone svetovidovi* has a body with a more compressed and narrower inter-orbital width. The main diagnostic characters and morphometric measurements of the captured specimens of *B. svetovidovi* are given in centimetres: the five specimens were 33.8-42.8 cm in total length and 32.5-41.3 cm in standard length, and 47.76-97.64 g in the total weight for Marmara Sea (Yalova) and 20.4-46.2 cm in total length and 18.6-43.7 cm in standard length, and 8.51-111.50 g in the total weight for Black Sea (Düzce-Akcakoca).

The meristic characters: Dorsal fin rays 15-18, anal fin rays 19-23, pectoral fin rays 10-13 and gill rakers number 38-49 for Marmara and Black Seas specimens. All the morphometric measurements and meristic characters of *Belone svetovidovi* from the Marmara and Black Sea regions are given in Table 1.

Table 1. Morphometric measurements (cm) and meristic characters of Belone svetovidovi were collected in the Marmara and Black Sea regions.

| **Measurements (cm)** | | |
|---|---|---|
| ***Characters*** | **Marmara Sea (Yalova)** | **Black Sea (Düzce-Akçakoca)** |
| ***Morphometric*** | **Range (Mean±SD) (n=5)** | **Range (Mean±SD) (n=5)** |
| Total length (TL) | 33.8-42.8 (37.08±3.46) | 20.4-46.2 (30±10.06) |
| Fork length (FL) | 33.2-42 (36.06±3.46) | 19.6-44.6 (29.06±9.69) |
| Standard length (SL) | 32.5-41.3 (35.94±3.36) | 18.6-43.7 (27.84±9.78) |
| Head length (HL) | 14.07-17.81 (15.43±1.44) | 8.49-19.23 (12.48±4.19) |
| Eye diameter (ED) | 1.29-1.64 (1.42±0.13) | 0.78-1.77 (1.14±0.38) |
| Body depth (BD) | 2.10-2.66 (2.30±0.21) | 1.27-2.88 (1.87±0.62) |
| Length of dorsal fin | 5.65-7.15 (6.19±0.57) | 3.41-7.72 (5.01±1.68) |
| Length of anal fin | 6.69-8.48 (7.34±0.68) | 4.04-9.15 (5.94±1.99) |
| Length of pectoral fin | 2.54-3.21 (2.78±0.25) | 1.53-3.47 (2.25±0.75) |
| Length of ventral fin | 1.94-2.45 (2.12±0.19) | 1.17-2.65 (1.72±0.33) |
| ***Meristic*** | | |
| Number of rays in dorsal fin (D) | 15-18 | 15-17 |
| Number of rays in pectoral fin (P) | 10-12 | 10-13 |
| Number of rays in ventral fin (V) | I+5 | I+5 |
| Number of rays in anal fin (A) | 20-22 | 19-23 |
| Gill rakers number (First-gill arch) | 39-49 | 38-48 |
| Vertebrae numbers | 72-76 | 72-78 |
| Teeth count (teeth within a section of the middle of the upper jaw equaling the eye diameter) | 13-20 | 14-21 |

After sequencing of the mtDNA COI gene region, there were 13 variables, 13 parsim-info and 557 conservative nucleotides, of which 93 were parsimony informative over 570 bp sequences. All sequences were submitted from Genbank and accession numbers were received as OR234691.1-OR234700.1.

The Neighbour-Joining (NJ) and Maximum Parsimony (MP) phylogenetic tree analyses were given in Figure 3 and Figure 4. NJ and ML trees indicated similar tree topologies. *B. belone* and *B.*

*svetovidovi* were separated from the two branches and the outgroup *Sphyraena sphyraena* was branched as a separate branch. According to NJ and MP trees, *B. Belone* and *B. svetovidovi* were clustered in different sets.

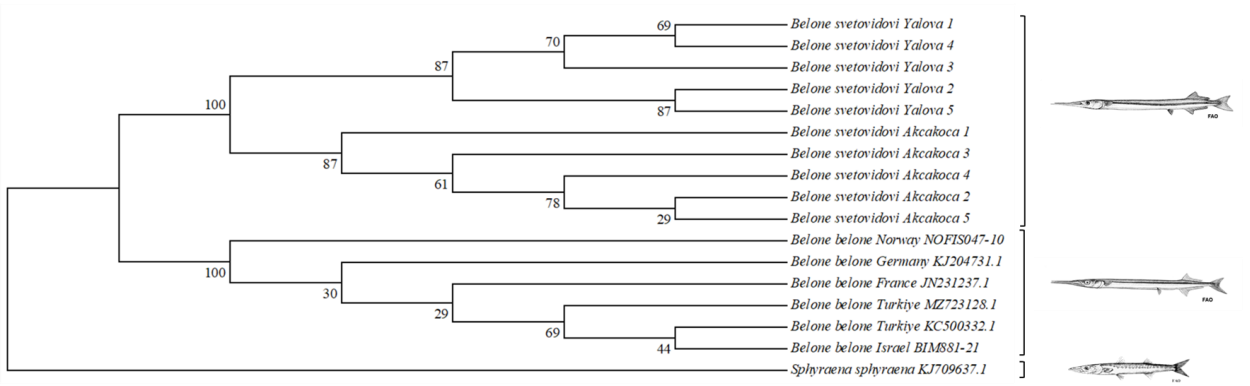


Figure 3. Neighbour-Joining tree (NJ) of garfish species with GenBank and Bold system sample references. The numbers above branches indicate bootstrap values among 1000 replicates. Fish sketches were given from FAO.

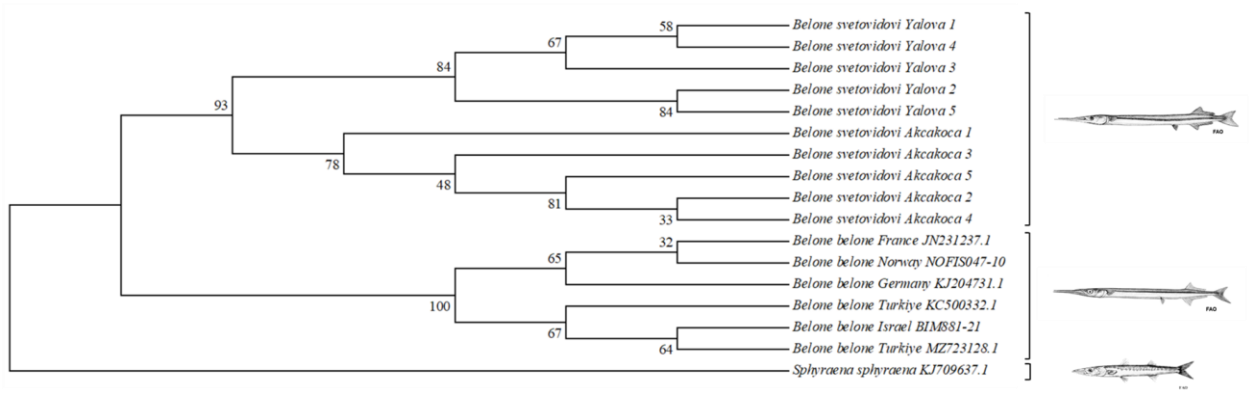


Figure 4. Maximum Parsimony tree (MP) of garfish species with GenBank and Bold system sample references. The numbers above branches indicate bootstrap values among 1000 replicates. Fish sketches were given from FAO.

**Discussion**

The phenotypic appearance of *B. sveovidovi* is quite similar to that of *Belone belone* and, in our belief, has thus far been confused with *B. belone*. The body is green or blue on the back and silvery white on the lower sides and belly of *B. svetovidovi*. At first glance, it differs from *B. belone* in the size and density of its beak teeth. This species can be distinguished from *B. belone* by the following characteristics: Its smaller, more delicate and features 13-21 teeth within a section of the middle of

the upper jaw equaling the diameter of its eye and teeth present on vomer and total gill rakers on first-gill arch 38-52 (Akşıray, 1987; Collette and Parin 1990; Dalyan and Eryılmaz, 2005).

In this study, general shape, morphometric measurements, meristic counts and colouration recorded in the present specimens are in total agreement with Collette and Parin (1990). All metric measurements and meristic data of garfish specimens are given in Table 1.

*Belone svetovidovi* was considered a synonym for *Belone belone* for many years. These two species are morphologically similar, which causes misidentification. We have revealed the genetic difference between these species by mtDNA COI sequencing analysis. The species of *B. belone* and *B. svetovidovi* concerned were separately clustered in our studies and the same has also been reported in previous studies on systematics and phylogeny of needlefishes using different mtDNA gene regions as 16s rRNA, Cyt B (Lovejoy, 2000; Lovejoy et al., 2004). The present and previous studies show that the COI gene has been used effectively in the genetic description of problematic species, and the mtDNA COI region provides a useful tool to identify species and to detect possibly cryptic species or new species (Turan et al., 2017; 2020; Ghouri et al., 2020; Doğdu and Turan, 2021).

In the present study, the first record of *B. svetovidovi* in the Marmara Sea and Black Sea and the first genetic and morphological confirmation of the garfish species in Türkiye are presented. New morphologic and genetic findings confirm the validity of including the short-beaked garfish *B. svetovidovi* in the Turkish ichthyofauna of the Marmara and Black Seas. The sample numbers obtained show that this species is widespread and settled in the waters of two sea regions of Turkey. Therefore, the Ministry of Agriculture and Forestry of Türkiye and the General Fishery Commission of the Mediterranean (GFCM) should consider validation of this species in management consideration and regulations since it is misidentified and considered as *Belone belone*. From our observations in the TÜBİTAK project (A holistic approach for bilateral sustainable exploitation of garfish (*Belone belone*) in Turkish and Bulgarian coastal waters: Genetic and morphological stock analyses methods coupled with data-limited stock assessment-121N777), *B. svetovidovi* is much more abundant than *B. belone* in the Black Sea.

**Acknowledgements**

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**Conflict of Interest**

The authors declare that they have no competing interests.

**Author Contributions**

C.T. performed all the experiments and drafted the main manuscript text. D.Y., S.A.D. M.G. and D.E. collected samples. C.T. and S.A.D. performed genetic analysis. C.T., D.Y., S.A.D., D.E., P.P.I. and V.S.R. contributed morfological analysis and drafting the manuscript. All authors reviewed and approved the final version of the manuscript.

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Natural and Engineering Sciences

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# Occurrence of the Scalloped Ribbonfish *Zu cristatus* (Lampridiformes) in the Gulf of Antalya, Türkiye

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## Abstract

The commercial trawler named "İdris Reis" caught a fish species that they had never seen or known before while catching shrimp in deep waters at the 36°25'04" N 30°37'17" E coordinates Gulf of Antalya on 04.04.2022. The species of fish caught was identified as *Zu cristatus* (Bonelli, 1819). With this study, one more species was added to the Gulf of Antalya fish fauna.

**Keywords:**

*Gulf of Antalya, scalloped ribbonfish, Zu cristatus*

**Article history:**

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## Introduction

The scalloped ribbonfish *Zu cristatus* (Bonelli, 1820) is a cosmopolitan mesobathypelagic species that occurs in tropical to temperate waters of the Atlantic and Indo-Pacific. *Z. cristatus* is a member of the Trachipteridae family which has 2 genera and 10 species, It has been reported that this fish inhibit waters at depht between 0-800 m (Quigley & Henderson 2014), the head swims up and its diet consists of small fishes and squids (Whitehead et al., 1984).

The majority of studies are based on records from the regions where the species is caught. For this reason, there is no detailed information about the biology of the fish. Studies on the biology of fish generally consist of studies on egg, larvae and fry. It has been reported that 16 individuals, mostly young juveniles, were caught between 1846 and 1973 in the Adriatic Sea (Jardas, 1980).

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According Dulcic (2002), the eggs and larvae of this fish in the Mediterranean were first described by Sanzo in 1918 and Sparta in 1956. Based on these descriptions, Dulcic (2002) also detected the eggs of fish in the Adriatic Sea.

There is no record of *Zu cristatus* being caught in Gulf of Antalya, on the Mediterranean coast of Turkey. This study constitutes the first occurance of the existence of *Zu cristatus* in the Gulf of Antalya. With the study, one more species has been added to the Gulf of Antalya fish fauna.

## Materials and Methods

### *Sampling and Morphological Analysis*

The commercial trawler named "İdris Reis" caught a fish species that they had never seen or known before while catching shrimp in deep waters (450 m) at the 36°25'04" N 30°37'17" E coordinates of Gulf of Antalya on 04.04.2022 (Figure 1). This fish was taken from fishermen and brought to the laboratory of Akdeniz University Fisheries Faculty. The species was identified and determined as *Zu cristatus*, which is rare in the Mediterranean.


Figure 1. The map of Gulf of Antalya where *Zu cristatus* is obtained.

First the fish was transported alive in a bucket with an air pump to the Antalya Fishing Shelter and then delivered to the laboratory of Faculty of Fisheries, Akdeniz University, Antalya. In lab metric and meristic characters of the captured fish were determined (Figure 2).

Figure 2. Scalloped Ribbonfish *Zu cristatus*, captured from Antalya, in the Gulf of Antalya/Mediterranean Sea, Turkey ( Photo by M. Gökoğlu).

**Results**

The fish species caught for the first time (on 04.04.2022) by a trawl net in the 450 m deep waters of Antalya Bay was determined as *Zu cristatus*. There are not any records of this fish species being caught in the Gulf of Antalya before.

In this study, the captured specimen was 72 cm. in total length, 59 cm. in standard length, and 490 g in total weight. Meristic data of Zu cristatus specimen were as follows: dorsal fin rays 130; anal fin III + 8; pectoral fin rays 15; pelvic fin rays 5; eye diameter 3,5 cm. There were about 8-9 dark vertical bars on the body.

**Discussion**

The scalloped ribbonfish *Zu cristatus* (Bonelli, 1820) (Pisces: Trachipteridae) is a meso-bathypelagic and cosmopolitan fish, inhabiting the Mediterranean Sea, Azores and Madeira in the Atlantic, Pacific and Indian Oceans (Dulcic, 2002). This fish is very rare in the Mediterranean. (Bianco et al., 2006). However, in the ichthyological literature, the existence of *Zu cristatus* has been confirmed and documented mainly in the Adriatic Sea, Ligurian Sea, Tyrrhenian Sea, the coasts of Spain and Algeria, and the Gulf of Tunisia (north of Tunisia) (Bradai & El Ouaer, 2012).

Bradai & El Ouaer (2012) also caught this fish on the Tunisian coast and determined the total length of the fish as 170 mm. Psomadakis et al., (2007) reported that *Zu cristatus* reaches a maximum length of 118 cm. These researchers had been caught two adult individuals with a total length and weight of 1219 mm, 2800 g and 1115 mm, 2160 g in the Gulf of Genoa. According to Quigley & Henderson (2014), *Z. cristatus* adults are found at depths of 150 - 800 m in summer, while young ones prefer shallower waters. In our study, fish were caught in the deep waters (450 m) of Antalya Bay. During the literature scanning on *Z. cristatus*, no record of this fish was reported from Antalya Bay. However, Gökoğlu & Özen (2021) reported *Trachipterus trachypterus* belonging to the Trachipteridae family from Gulf of Antalya.

In this study, *Zu cristatus* was first reported from the Gulf of Antalya and another species was added to the Gulf of Antalya fish fauna

**Conflict of Interest**

The authors declare that they have no competing interests.

**Author Contributions**

E.B. got this materal and M.G., F.Ç. and A.Y. is written this paper.

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Natural and Engineering Sciences

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# Investigation of Groundwater Zooplankton Fauna from Water Wells in Kilis Province from Türkiye

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## Abstract

A total of 27 taxons, 12 from Rotifera, 1 from Cladocera, and 14 from Copepoda, were determined in the study, which was conducted by sampling 4 times from 29 water wells. A total of 3 families were detected from Rotifera and Lecanidae was the richest family with 8 species. Among the 6 families of Copepoda, Cyclopoidae had 8 species. The rotifer species with the largest distribution areas were *Lecane closterocerca* (found in 15 wells), *Pleuroxus aduncus*, the only species from Cladocera, was found in 21 wells and *Kinnecaris xanthi* had the widest distribution area (found in 27 wells). In terms of total zooplankton species, it was determined that wells 3, 12, and 18 were the richest with 14 species. While Rotifera was found in limited quantities in all water wells, *Pleuroxus aduncus* from Cladocera, *Diacyclops longuioides*, *Megacyclops viridis*, *Monchenkocyclops mehmetadami* and *Thermocyclops dybowski* from Copepoda were found in very large quantities. In addition, the genus *Ectinosoma* is reported for the first time from inland waters of Türkiye with this study.

**Keywords:**

*Zooplankton, water wells, Kilis province*

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## Introduction

Groundwater covers a wide region, accounting for roughly 40% of global inland waterways (Castany, 1982), and is made up of a variety of habitats that are more or less interconnected (Botosaneanu, 1986; Gibert et al., 1994; 1997; Palmer et al., 1997). Stygobionts, stygophiles, and stygoxenes are three types of zooplankton that are classed based on their degree of adaptability to

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groundwater living. Throughout their entire life cycle, stygobionts are inextricably linked to the groundwater environment, and they frequently adapt to its biotic and abiotic conditions. Stygophiles can live and reproduce in both underground and epigean marginal habitats, including springs, edaphic habitats, near-surface sediments of running waterways, and lentic water bodies; additionally, they may or may not contain incipient troglomorphic traits. Furthermore, the stygophile state should not always be viewed as a transitional evolutionary stage in the'stigobization' process (Stoch, 1995).

Animals thrive in aquifer pore gaps and cracks. Crustaceans make up the majority of animals, both in terms of quantity and species diversity. The biodiversity of groundwater is now clearer than previously thought. Inland water groundwater environments have evolved into three primary types of aquifers: karst, fissured, and porous, though these classifications are not always clearly defined in natural conditions. Furthermore, groundwater is closely associated with lentic water basins, stream channels, and springs through groundwater below and lateral to surface open waters. These so-called "dynamic transition zones" (Stanford & Ward 1993; Gibert et al., 1994; 1997) are locations where surface and groundwater systems interact (Vervier et al., 1993).

Meiofauna includes the majority of groundwater creatures. Meiofauna has a usual size range of 0.3-1 mm. The groundwater fauna has morphologically adapted to the subsurface habitat by maximizing the limited dwelling areas in the pores. For millions of years, groundwater has been one of the world's oldest habitats, with generally stable environmental conditions. However, even in Central and Southern Europe, where the study is rather extensive, groundwater biodiversity research is still in its infancy, and new species are continuously being identified.

The spatial distribution of fauna is often quite unequal due to the heterogeneity in groundwater (in terms of organic matter distribution, oxygen, and matrix pore size). Groundwater fauna has a localized uneven distribution, indicating subterranean habitat heterogeneity. Furthermore, it suggests that true groundwater fauna (stygobionts) have adapted well to the unique living conditions in groundwater. As a result, groundwater fauna can be utilized to assess groundwater ecology.

Copepods, one of the most important groundwater fauna components, have successfully infiltrated subsurface habitats in both marine and territorial waters at various times and in various ways. Stygobionts are found in six of the ten known Copepoda orders: Platycopioida, Calanoida, Misophrioida, Cyclopoida, Harpacticoida, and Gelyelloida. About 897 species and subspecies of Cyclopoida and Harpacticoida, chiefly belonging to the Canthocamptidae, Parastenocarididae, and Ameiridae, including the cyclopoid family Cyclopidae, successfully colonized inland groundwater.

The fauna of groundwater comprises nearly all of the major taxonomic groupings found in limnic surface waters, although it has not been fully researched thus far. This research was carried

out to determine the groundwater fauna in Türkiye, which had previously been done in small quantities.

## Materials and Methods

Zooplankton samples were collected by vertical hauls using a 60 µm mesh size plankton net from 29 different water wells within the borders of Kilis Province in February 2019, June 2019, September 2019, and July 2020 (Figure 1).

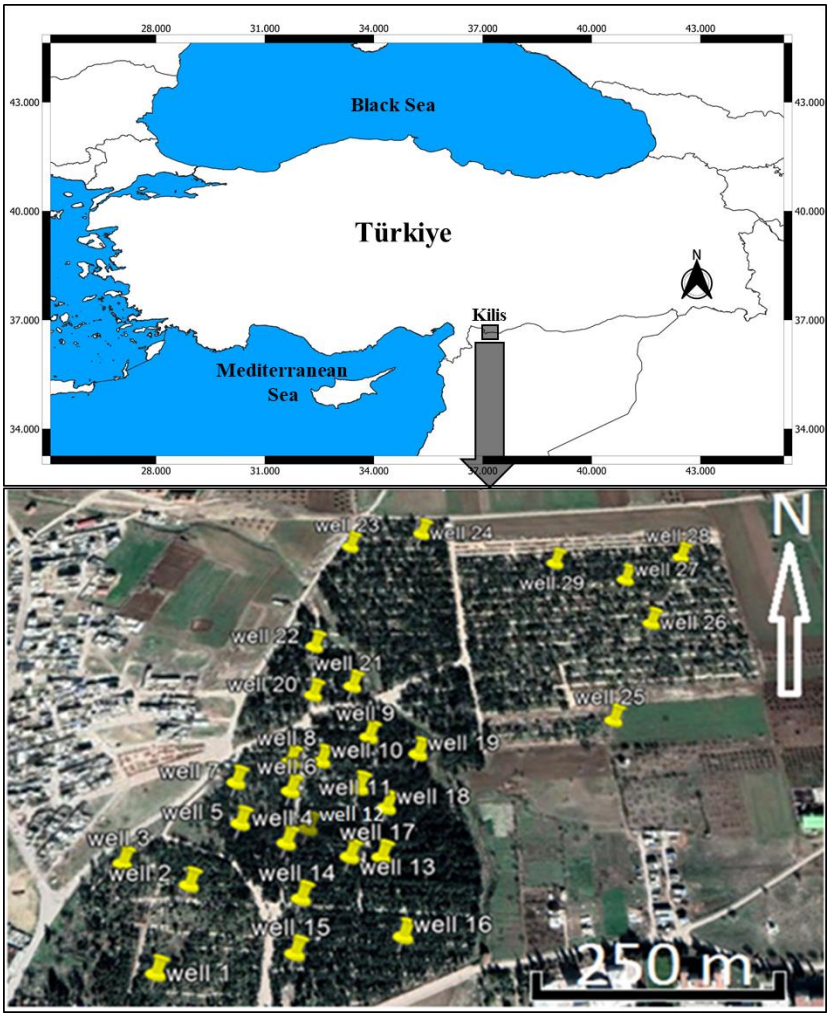


Figure 1. Sampling points of zooplankton.

A 0.5 kg metal weight was attached to the collector of the net and the net was lowered to the bottom of the well. The net was used to stir the well water vigorously to enable proper mixing of the zooplankton in the benthic layers with the water. The net was then raised and 10 replicates were made for each well. The coordinates of the sampling wells, the depth of the wells from the surface to the bottom, the water depth at the time of sampling, and the widths of the wells are given in Table 1.

Table 1. Coordinates, depth, width, and water depth of wells.

| Sampling stations | Latitude | Longitude | Well depth (m) | Water depth (m) | Well width (m) |
|---|---|---|---|---|---|
| Well 1 | 36° 43′ 07.64″ N | 37° 07′ 44.79″ E | 14 | 5 | 1.90 |
| Well 2 | 36° 43′ 11.38″ N | 37° 07′ 45.68″ E | 13 | 7 | 1.80 |
| Well 3 | 36° 43′ 12.34″ N | 37° 07′ 42.56″ E | 13 | 7 | 2.00 |
| Well 4 | 36° 43′ 13.27″ N | 37° 07′ 49.84″ E | 12 | 4 | 1.40 |
| Well 5 | 36° 43′ 14.18″ N | 37° 07′ 47.64″ E | 12 | 6 | 1.20 |
| Well 6 | 36° 43′ 15.72″ N | 37° 07′ 49.81″ E | 10 | 4 | 1.10 |
| Well 7 | 36° 43′ 16.14″ N | 37° 07′ 47.22″ E | 12 | 6 | 1.20 |
| Well 8 | 36° 43′ 17.15″ N | 37° 07′ 49.66″ E | 5 | 2 | 2.10 |
| Well 9 | 36° 43′ 18.41″ N | 37° 07′ 53.32″ E | 9 | 5 | 2.10 |
| Well 10 | 36° 43′ 17.18″ N | 37° 07′ 51.13″ E | 12 | 4.5 | 2.00 |
| Well 11 | 36° 43′ 15.86″ N | 37° 07′ 52.99″ E | 9 | 5 | 2.00 |
| Well 12 | 36° 43′ 13.94″ N | 37° 07′ 50.76″ E | 11 | 4 | 1.60 |
| Well 13 | 36° 43′ 12.65″ N | 37° 07′ 52.76″ E | 13 | 7 | 1.50 |
| Well 14 | 36° 43′ 10.79″ N | 37° 07′ 50.72″ E | 13 | 5 | 1.40 |
| Well 15 | 36° 43′ 08.46″ N | 37° 07′ 50.65″ E | 12 | 2 | 1.20 |
| Well 16 | 36° 43′ 09.17″ N | 37° 08′ 55.29″ E | 12 | 4 | 1.10 |
| Well 17 | 36° 43′ 12.62″ N | 37° 07′ 54.19″ E | 14 | 5 | 1.20 |
| Well 18 | 36° 43′ 14.92″ N | 37° 07′ 54.32″ E | 9 | 5 | 1.60 |
| Well 19 | 36° 43′ 17.55″ N | 37° 07′ 55.71″ E | 7 | 2 | 1.90 |
| Well 20 | 36° 43′ 20.61″ N | 37° 07′ 50.47″ E | 15 | 7 | 1.70 |
| Well 21 | 36° 43′ 21.09″ N | 37° 07′ 52.37″ E | 11 | 6 | 1.80 |
| Well 22 | 36° 43′ 23.29″ N | 37° 07′ 50.33″ E | 13 | 4 | 1.60 |
| Well 23 | 36° 43′ 29.28″ N | 37° 07′ 51.77″ E | 11 | 6 | 1.50 |
| Well 24 | 36° 43′ 30.10″ N | 37° 07′ 55.56″ E | 12 | 7 | 1.80 |
| Well 25 | 36° 43′ 19.27″ N | 37° 08′ 05.13″ E | 16 | 7 | 2.10 |
| Well 26 | 36° 43′ 24.62″ N | 37° 08′ 07.44″ E | 12 | 4 | 1.90 |
| Well 27 | 36° 43′ 27.22″ N | 37° 08′ 06.32″ E | 11 | 2 | 1.70 |
| Well 28 | 36° 43′ 28.61″ N | 37° 08′ 09.37″ E | 17 | 5 | 2.20 |
| Well 29 | 36° 43′ 28.24″ N | 37° 08′ 02.67″ E | 12 | 6 | 1.80 |

The zooplankton samples were fixed and kept in 4% formaldehyde after sampling and then analyzed in a mixture of distilled water and glycerol. The general abundance of zooplankton was examined rather than the counting method in a quantitative zooplankton study. Absent (-), very little (*), little (+), abundant (++), and extremely abundant (+++) were the scores. An inverted microscope was used to view zooplankton species, which were then identified using a binocular microscope (Olympus CH40). The specimens were identified and examined using Borutsky (1964), Scourfield & Harding (1966), Dussart (1969), Damian-Georgescu (1970), Ruttner-Kolisko (1974), Smirnov (1974), Kiefer (1978), Koste (1978), Negrea (1983), Korinek (1987), Segers (1995), and Galassi & De Laurentiis (2004).

**Results**

In this study, 14 copepods (51.85%), 12 rotifers (44.45%), and 1 cladoceran (3.70%) were recorded in the water wells (Table 2). Rotifera, which was determined to have three different families, Lecanidae has the most species with eight species, followed by the families Lepadellidae and Notammatidae, both of which have two species. Cladocera was represented by a species belonging to the Chydoridae family. While Copepoda had eight species in Cyclopoidae, Canthocamptidae had two; Ameiridae, Parastenocarididae, Ectinosomatidae, and Phyllognathopodidae each had one species (Table 2).

Table 2. Identified zooplankton species

| | |
|---|---|
| **Rotifera** | |
| Notommatidae | *Cephalodella forficula* (Ehrenberg, 1838) |
| | *Cephalodella gibba* (Ehrenberg, 1838) |
| Lepadellidae | *Colurella uncinata* (Müller, 1773) |
| | *Lepadella patella* (Müller,1786) |
| Lecanidae | *Lecane bulla* (Gosse,1886) |
| | *Lecane closterocerca* (Schmarda, 1859) |
| | *Lecane flexilis* (Gosse,1886) |
| | *Lecane hamata* (Stokes,1896) |
| | *Lecane inermis* (Bryce,1892) |
| | *Lecane ludwigi* (Eckstein,1893) |
| | *Lecane pyriformis* (Daday,1905) |
| | *Lecane tenuiseta* Harring, 1914 |
| **Cladocera** | |
| Chydoridae | *Pleuroxus aduncus* (Jurine, 1820) |
| **Copepoda** | |
| Cyclopidae | *Diacyclops languidoides* (Lilljeborg, 1901) |
| | *Eucyclops serrulatus* (Fischer, 1851) |
| | *Megacyclops viridis* (Jurine, 1820) |
| | *Microcyclops* sp. |
| | *Monchenkocyclops mehmetadami* Karaytuğ, Bozkurt &Sönmez, 2018 |
| | *Paracyclops chiltoni* (Thomson, 1883) |
| | *Thermocyclops dybowski* (Lande, 1890) |
| | *Tropocyclops prasinus* (Fischer, 1860) |
| Canthocamptidae | *Attheyella crassa* (Sars,1863) |
| | *Elaphoidella* sp. |
| Ameiridae | *Nitocrella stammeri* Chappuis, 1938 |
| Parastenocarididae | *Kinnecaris xanthi* Bruno & Cottarelli, 2015 |
| Ectinosomatidae | *Ectinosoma* sp. |
| Phyllognathopodidae | *Phyllognathopus viguieri* (Maupas, 1892) |

According to Table 3, the rotifer species with the largest distribution areas were *Lecane closterocerca* (found in 15 wells), *L. pyriformis* (13 wells), and *L. hamata* (12 wells). *Pleuroxus aduncus*, the only species from Cladocera, was found in 21 wells. *Kinnecaris xanthi* had the widest distribution area (found in 27 wells), followed by *D. longuioides* and *M. mehmetadami* (25 wells), and *Nitocrella stammeri* (23 wells). Some zooplankton species in the study were selective to their environmental conditions, showing limited distribution, and were recorded in very few wells. *Cephalodella gibba*, *C. uncinata*, *Lecane bulla*, and *L. ludwigi* from Rotifera; *Microcyclops* sp. and *Phyllognathopus viguieri* from Copepoda were recorded in one well each (Table 3). Most species (5 species) from Rotifera were recorded in wells 14, 15, and 25 followed by wells 3, 5, 19, 20, and 26 with 4 species and wells 2, 4, 8, 10, 12, 16, 18 and 27 with 3 species. The most species from Copepoda were recorded in wells 12 and 18 (10 species), followed by 9 species in wells 3, 5, and 8, and 8 species in wells 7, 9, 10, 19, and 27 (Table 3). In terms of total zooplankton species, wells 3, 12, and 18 were the richest with 14 species, followed by wells 5, 14, 19, and 25 with 13 species and well 8, 10, 15, 26, and 27 with 12 species (Table 3). While the wells were rich in the variety of rotifer and copepod, they were very poor in terms of cladoceran. On the other hand, while no rotifers were recorded in wells 6, 11, 21, 28, and 29, only one rotifer species was recorded in wells 9, 17, 22, 23, and 24. It was determined that the wells with 2 species from Copepoda and the least species were recorded in wells 11 and 29 (Table 3).

Table 3. Determined zooplankton species in different water wells.

| **Species / Wells** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rotifera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *C. forficula* | - | - | - | + | - | - | + | - | - | - | - | - | + | - | + | + | - | - | + | - | - | - | - | + | + | - | + | - | - | **9** |
| *C. gibba* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | **1** |
| *C. uncinata* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **1** |
| *L. bulla* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **1** |
| *L. closterocerca* | - | - | + | + | + | - | + | + | - | + | - | + | - | + | + | + | - | + | + | - | - | - | + | - | + | - | + | - | - | **15** |
| *L. flexilis* | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | **2** |
| *L. hamata* | + | + | + | - | + | - | - | - | + | + | - | + | - | + | - | - | - | - | + | + | - | - | - | - | + | + | - | - | - | **12** |
| *L. inermis* | - | - | + | - | - | - | - | - | - | - | - | - | - | + | + | - | + | + | - | + | - | + | - | - | + | | - | - | - | **8** |
| *L. ludwigi* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | **1** |
| *L. pyriformis* | + | + | + | + | + | - | - | + | - | + | - | - | + | + | - | - | - | - | + | + | - | - | - | - | + | + | - | - | - | **13** |
| *L. tenuiseta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | **2** |
| *L. patella* | - | + | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | **5** |
| **Total rotifer** | **2** | **3** | **4** | **3** | **4** | **0** | **2** | **3** | **1** | **3** | **0** | **3** | **2** | **5** | **5** | **3** | **1** | **3** | **4** | **4** | **0** | **1** | **1** | **1** | **5** | **4** | **3** | **0** | **0** | |
| **Cladocera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. aduncus* | - | + | + | + | - | + | - | - | - | + | - | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | - | **21** |
| **Copepoda** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D. languidoides* | - | + | + | + | + | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | **25** |
| *E. serrulatus* | - | - | + | + | + | - | + | + | + | + | - | + | - | - | - | - | + | + | - | - | - | - | - | - | + | + | + | + | - | **14** |
| *M. viridis* | + | - | + | - | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | - | - | + | - | + | + | + | + | - | + | **21** |
| *Microcyclops* sp. | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **1** |
| *M. mehmetadami* | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | - | **25** |
| *P. chiltoni* | + | - | + | + | - | - | + | - | + | + | - | + | - | + | - | - | - | + | - | - | - | - | + | - | - | + | - | - | - | **11** |
| *T. dybowski* | - | + | + | - | + | - | - | + | - | - | - | + | - | + | - | - | - | + | + | - | + | - | - | + | - | - | + | - | - | **11** |
| *T. prasinus* | + | - | - | + | + | + | + | - | - | + | - | - | - | - | - | - | + | - | + | - | + | + | + | - | - | - | - | - | - | **11** |
| *A. crassa* | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | + | + | - | - | - | - | - | - | - | **5** |
| *Ectinosoma* sp. | - | - | + | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | **4** |
| *Elaphoidella* sp. | - | - | + | - | - | - | - | + | + | - | - | + | - | + | + | - | - | + | - | - | - | - | + | - | - | - | + | - | - | **9** |
| *K. xanthi* | - | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | **27** |
| *N. stammeri* | - | + | - | + | + | + | + | + | + | + | - | + | + | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | - | **23** |
| *P. viguieri* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | **1** |
| **Total copepod** | **3** | **5** | **9** | **7** | **9** | **3** | **8** | **9** | **8** | **8** | **2** | **10** | **5** | **7** | **6** | **5** | **7** | **10** | **8** | **4** | **7** | **6** | **7** | **6** | **7** | **7** | **8** | **5** | **2** | |
| **Total zooplankton** | **5** | **9** | **14** | **11** | **13** | **4** | **10** | **12** | **9** | **12** | **2** | **14** | **8** | **13** | **12** | **9** | **9** | **14** | **13** | **9** | **8** | **7** | **9** | **8** | **13** | **12** | **12** | **6** | **2** | |

+: Available, -: absent

Seven of the 12 species from Rotifera, one species from Cladocera, and 12 of 14 species from Copepoda were recorded in different seasons and at varying levels of abundance in wells. *L. closterocerca* from Rotifera at the first sampling time (24.02.2019) in wells 18 and 19; at the third sampling time (15.09.2019) in wells 5 and 16, and at the last sampling time (05.07.2020) in wells 3 were abundant (++). While *L. hamata* was abundant in well 19 at the second and fourth sampling times, *L. pyriformis* was abundant in well 10 at the third sampling time. *P. aduncus* from Cladocera was recorded as very abundant (+++) in well 25 in the first sampling, while it was recorded as abundant in wells 4, 16, and 19. In the second sampling, it was recorded as very abundant in well 15 and abundant in well 16. In the third sampling, it was abundant in wells 13 and 16, while it was abundant in well 18. In the last sampling, it was determined that they were abundant in wells 4, 16, 19, and 25 (Table 4). *M. mehmetadami* from Copepoda was very abundant in well 12 in the last sampling. In that first sampling, in wells 5 and 7; In the second sampling, in wells 19, 23, 24, and 26; in the third sampling wells 5, 7, 21, 23, and 25; in the last sampling, it was abundant in wells 3, 4, 5, 7, 11, 14, 19, 21, 24, 25 and 27. *E. serrulatus* from Copepoda was abundant in well 7 in the second sampling and well 28 in the third sampling. While *D. longuioides* from Copepoda were abundant in wells 9 and 19 in the third sampling, they were abundant in wells 4, 7, 13, 18, 21, 23, and 25. In the fourth sampling, they were abundant in wells 2, 4, 8, 19, 23, and 25. In the second sampling, *M. viridis* from Copepoda was abundant in well 11; in the third sampling, while *M. viridis* was very abundant in well 10, it was also abundant in wells 2 and 17. In the fourth sampling, It was abundant in well 29.

*P. chiltoni* was abundant in well 7 in the first sampling, abundant in well 1 in the second and third samplings, and abundant in wells 1, 7, and 26 in the final sampling. *T. dybowski* was very abundant in well 5 in the third sampling, but it was abundant in well 18. In the second sampling, *T. prasinus* was very abundant in wells 17 and 19, and abundant in well 21. In the third sampling, it was very abundant in well 5 and abundant in well 18. In the last sampling, it was abundant only in well number 19. While *Elaphoidella* sp. was abundant in well 27 in the last sampling, *K. xanthi* was abundant in wells 9, 21, and 24 in the second sampling (Table 4).

Table 4. Zooplankton in water wells according to sampling times

| **Species** **Wells** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rotifera** | | | | | | | | | | | | | | | **24.02.2019** | | | | | | | | | | | | | | |
| *C. forficula* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | * | - | - | - | - | * | - | - | - | - | - |
| *C. gibba* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - |
| *C. uncinata* | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. bulla* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. closterocerca* | - | - | * | * | + | - | * | * | - | + | - | * | - | - | * | + | - | ++ | ++ | - | - | - | - | - | + | - | + | - | - |
| *L. flexilis* | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| *L. hamata* | * | - | * | - | - | - | - | - | * | + | - | - | - | * | - | - | - | - | + | * | - | - | - | - | * | * | - | - | - |
| *L. inermis* | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | * | | - | - | - |
| *L. ludwigi* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - |
| *L. pyriformis* | - | * | - | - | * | - | - | - | - | * | - | - | * | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - |
| *L. tenuiseta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. patella* | - | * | - | - | + | - | - | - | - | - | - | * | - | - | - | - | - | - | - | * | - | - | - | - | - | * | - | - | - |
| **Cladocera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. aduncus* | - | * | + | + + | - | * | - | - | - | * | - | - | + | + | + | ++ | * | * | + + | * | * | - | * | * | ++ + | - | * | * | - |
| **Copepoda** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D. languidoides* | - | - | - | - | * | - | + | + | + | * | - | + | * | + | * | + | * | + | * | + | + | + | + | - | * | * | + | + | - |
| *E. serrulatus* | - | - | - | - | - | - | | + | - | - | - | - | - | - | - | - | * | + | - | - | - | - | - | - | - | * | - | + | - |
| *M. viridis* | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | + |
| *M. mehmetadami* | - | - | + | * | ++ | - | ++ | * | + | + | + | + | + | + | + | - | * | + | * | - | + | + | + | + | + | + | - | + | - |
| *P. chiltoni* | + | - | - | - | - | - | ++ | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *T. dybowski* | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - |
| *T. prasinus* | + | - | - | - | - | * | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| *A. crassa* | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | * | - | * | + | - | - | - | - | - | - | - |
| *Elaphoidella* sp. | - | - | - | - | - | - | - | * | + | - | - | * | - | - | * | - | - | * | - | - | - | - | * | - | - | - | + | - | - |
| *K. xanthi* | - | - | * | * | * | - | * | * | + | * | - | + | + | * | * | + | - | - | * | - | - | * | - | + | * | + | - | - | * |
| *N. stammeri* | - | + | - | - | - | * | - | + | * | - | - | * | * | - | - | * | * | - | - | * | - | - | - | * | * | * | + | * | - |
| **Rotifera** | | | | | | | | | | | | | | | **30.06.2019** | | | | | | | | | | | | | | |
| *C. forficula* | - | - | - | * | - | - | - | - | - | - | - | - | * | - | * | * | - | - | - | - | - | - | - | * | - | - | * | - | - |
| *L. closterocerca* | - | - | - | * | + | - | - | * | - | + | - | - | - | - | + | + | - | - | - | - | - | - | * | - | - | - | + | - | - |
| *L. hamata* | * | * | * | - | * | - | - | - | * | + | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | - |
| *L. inermis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | + | - | * | - | - | - | - | - | - | - |
| *L. ludwigi* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - |
| *L. pyriformis* | * | * | + | * | * | - | - | * | - | * | - | - | * | * | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - |
| *L. tenuiseta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. patella* | - | - | - | - | * | - | - | - | - | - | - | * | - | - | - | - | - | - | - | * | - | - | - | - | - | * | - | - | - |

Table 4. Contiuned

| Species Wells | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cladocera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. aduncus* | - | - | - | - | - | * | - | - | - | - | - | - | + | - | ++ + | ++ | * | + | * | - | * | - | - | * | - | - | * | * | - |
| **Copepoda** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D. languidoides* | - | + | - | * | * | - | + | + | + | + | - | * | * | + | - | - | - | + | - | - | - | - | - | - | - | * | - | - | - |
| *E. serrulatus* | - | - | - | - | - | - | ++ | - | + | - | - | * | - | - | - | - | - | * | - | - | - | - | - | - | * | - | - | + | - |
| *M. viridis* | - | - | * | - | * | - | - | + | - | - | ++ | * | - | - | + | * | - | - | - | - | - | * | - | * | - | + | - | - | + |
| *Microcyclops* sp. | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *M. mehmetadami* | - | - | * | + | + | - | * | + | + | + | - | * | * | * | * | - | + | + | ++ | + | + | + | ++ | ++ | + | ++ | - | + | - |
| *P. chiltoni* | ++ | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *T. dybowski* | - | - | - | - | + | - | - | * | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | * | - | - |
| *T. prasinus* | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | ++ + | | ++ + | - | ++ | - | - | - | - | - | - | - | - |
| *A. crassa* | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | * | * | - | * | - | - | - | - | - | - | - | - |
| *Ectinosoma* sp. | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Elaphoidella* sp. | - | - | - | - | - | - | - | * | + | - | - | * | - | - | - | - | - | - | - | - | - | - | * | - | - | - | + | - | - |
| *K. xanthi* | - | * | * | * | * | - | * | * | ++ | * | - | + | + | * | * | * | | + | - | + | ++ | * | + | ++ | + | + | + | | * |
| *N. stammeri* | - | * | - | - | - | * | * | * | * | + | - | - | - | - | - | - | * | * | - | * | - | - | - | - | - | * | + | * | - |
| **Rotifera** | | | | | | | | | | | | | | | **15.09.2019** | | | | | | | | | | | | | | |
| *C. forficula* | - | - | - | - | - | - | * | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | * | * | - | - | - | - |
| *C. uncinata* | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. closterocerca* | - | - | * | + | ++ | - | * | * | - | * | - | * | - | - | * | ++ | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. hamata* | - | - | - | - | - | - | - | - | * | - | - | - | - | * | - | - | - | - | * | * | - | - | - | - | - | * | - | - | - |
| *L. inermis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | * | - | - | - | - |
| *L. pyriformis* | - | * | - | - | * | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - | * | - | - | - | - | * | + | - | - | - |
| *L. tenuiseta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | | - | - | - | - | - | - | - | - | - |
| *L. patella* | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | * | - | - | - |
| **Cladocera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. aduncus* | - | * | * | - | - | * | - | - | - | * | - | * | ++ + | * | * | ++ + | - | ++ | + | * | * | - | * | - | * | * | * | * | - |
| **Copepoda** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D. languidoides* | - | + | * | + + | - | - | ++ | * | +++ | * | - | + | ++ | + | * | + | * | ++ | ++ + | + | ++ | * | ++ | + | ++ | * | + | + | - |
| *E. serrulatus* | - | - | - | - | - | - | + | - | * | * | - | - | - | - | - | - | * | * | - | - | - | - | - | - | * | * | - | ++ | - |
| *M. viridis* | - | + + | - | - | - | - | - | - | - | +++ | * | - | * | + | * | - | ++ | - | * | - | - | - | - | - | * | * | - | - | + |
| *M. mehmetadami* | - | * | * | - | ++ | - | ++ | * | + | - | + | + | + | - | * | - | * | * | - | - | ++ | - | ++ | * | ++ | - | + | + | - |
| *P. chiltoni* | ++ | - | * | + | - | - | * | - | * | * | - | - | - | * | - | - | - | * | - | - | - | - | + | - | - | - | - | - | - |
| *T. dybowski* | - | * | + | - | ++ + | - | - | * | - | - | - | * | - | * | - | - | - | ++ | + | - | * | - | - | * | - | - | * | - | - |
| *T. prasinus* | ++ | - | - | * | - | * | - | - | - | * | - | - | - | - | - | - | - | - | ++ | - | * | * | + | - | - | - | - | - | - |

Table 4. Contiuned

| **Species Wells** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **29** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. crassa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | * | - | - | - | - | - | - | - | - |
| *Ectinosoma* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - |
| *Elaphoidella* sp. | - | - | * | | - | - | - | * | + | - | - | - | - | * | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - |
| *K. xanthi* | - | - | * | + | * | * | - | - | + | * | - | * | + | * | * | + | + | * | + | * | * | * | - | + | * | * | + | * | * |
| *N. stammeri* | - | - | - | - | - | - | - | * | - | - | - | * | - | - | * | * | * | - | + | * | * | - | + | * | * | * | + | - | - |
| **Rotifera** | | | | | | | | | | | | | | | **05.07.2020** | | | | | | | | | | | | | | |
| *C. forficula* | - | - | - | - | - | - | - | - | - | - | - | - | * | - | * | * | - | - | - | - | - | - | - | * | - | - | * | - | - |
| *C. gibba* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - |
| *L. closterocerca* | - | - | + | + | * | - | - | + | - | * | - | * | | * | * | + | | | | | | | - | - | - | - | + | - | - |
| | | | + | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *L. flexilis* | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. hamata* | - | - | - | - | - | - | - | - | * | - | - | * | - | * | - | - | - | - | + | - | - | - | - | - | * | * | - | - | - |
| | | | | | | | | | | | | | | | | | | | + | | | | | | | | | | |
| *L. inermis* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | * | * | - | - | - | * | - | - | * | - | - | - | - |
| *L. pyriformis* | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | * | * | - | - | - | - | - | - | - | - | - |
| *L. tenuiseta* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *L. patella* | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | - | - | - | - | * | - | - | - |
| **Cladocera** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. aduncus* | - | - | - | + | - | * | - | - | - | * | - | - | + | + | + | ++ | * | - | + | * | * | - | * | * | ++ | - | - | - | - |
| | | | | + | | | | | | | | | | | | | | | + | | | | | | | | | | |
| **Copepoda** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *D.languidoides* | - | + | - | + | + | - | * | ++ | + | * | - | - | * | - | * | + | * | + | + | + | + | + | ++ | - | ++ | - | - | + | - |
| | | + | | + | | | | | | | | | | | | | | | + | | | | | | | | | | |
| *E. serrulatus* | - | - | + | + | * | - | - | + | + | - | - | - | - | - | - | - | * | * | - | - | - | - | - | - | * | - | * | - | - |
| *M. viridis* | + | - | - | - | - | - | * | - | - | - | * | * | - | - | + | - | - | + | - | - | - | - | - | * | * | * | * | - | ++ |
| *M. mehmetadami* | - | - | + | + | ++ | - | + | - | + | - | ++ | ++ | * | + | * | - | * | + | + | - | + | + | - | + | ++ | + | ++ | - | - |
| | | | + | + | | | + | | | | | + | | + | | | | | + | | + | | | + | | | | | |
| *P. chiltoni* | ++ | - | + | - | - | - | + | - | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - |
| | | | | | | | + | | | | | | | | | | | | | | | | | | | | | | |
| *T. dybowski* | - | + | * | * | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *T. prasinus* | - | - | - | - | + | - | * | - | - | * | - | - | - | - | - | - | - | - | + | - | * | - | + | - | - | - | - | - | - |
| | | | | | | | | | | | | | | | | | | | + | | | | | | | | | | |
| *A. crassa* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | * | - | * | - | - | - | - | - | - | - | - |
| *Ectinosoma* sp. | - | - | + | - | - | - | * | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Elaphoidella* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - |
| *K. xanthi* | - | - | + | * | * | * | * | - | + | * | - | * | + | * | * | + | - | * | + | + | + | * | + | + | * | * | * | * | * |
| *N. stammeri* | - | + | - | + | * | - | - | - | * | - | - | - | * | - | * | * | * | * | - | * | * | - | + | - | * | + | - | * | - |

-: Absent, *: very few, +: few, ++: abundant, +++: very abundant.

*Cephalodella forficula, Lecane closterocerca, L. hamata, L. inermis, L. pyriformis, L. tenuiseta, Lepadella patella, P. aduncus, D. longuioides, E. serrulatus, M. viridis, M. mehmetadami, P. chiltoni, T. dybowski, T. prasinus, A. crassa, Elaphoidella* sp., *K. xanthi* and *N. stammeri* were present at all sampling times but *L. bulla, Microcyclops sp.* and *P. viguieri* were recorded only once during the different sampling time (Table 5).

Table 5. The abundance of species by sampling time.

| | **Sampling Time** | | | |
|---|---|---|---|---|
| | **24.02. 2019** | **30.06.2019** | **15.09. 2019** | **05.07.2020** |
| **Rotifera** | | | | |
| *Cephalodella forficula* | * | * | * | * |
| *Cephalodella gibba* | * | - | - | * |
| *Colurella uncinata* | * | - | * | - |
| *Lecane bulla* | * | - | - | - |
| *Lecane closterocerca* | ++ | + | ++ | ++ |
| *Lecane flexilis* | + | - | - | * |
| *Lecane hamata* | + | ++ | * | ++ |
| *Lecane inermis* | + | + | * | + |
| *Lecane ludwigi* | * | * | - | - |
| *Lecane pyriformis* | * | + | ++ | * |
| *Lecane tenuiseta* | + | * | * | * |
| *Lepadella patella* | + | + | + | * |
| **Cladocera** | | | | |
| *Pleuroxus aduncus* | +++ | +++ | +++ | ++ |
| **Copepoda** | | | | |
| *Diacyclops languidoides* | ++ | + | +++ | ++ |
| *Eucyclops serrulatus* | + | ++ | ++ | + |
| *Megacyclops viridis* | + | ++ | +++ | ++ |
| *Microcyclops* sp. | - | * | - | - |
| *Monchenkocyclops mehmetadami* | ++ | ++ | ++ | +++ |
| *Paracyclops chiltoni* | ++ | ++ | ++ | ++ |
| *Thermocyclops dybowski* | + | + | +++ | + |
| *Tropocyclops prasinus* | + | +++ | ++ | ++ |
| *Attheyella crassa* | + | * | * | * |
| *Ectinosoma* sp. | - | * | * | + |
| *Elaphoidella* sp. | + | + | + | ++ |
| *Kinnecaris xanthi* | + | ++ | + | + |
| *Nitocrella stammeri* | + | + | + | + |
| *Phyllognathopus viguieri* | - | * | - | - |

-: Absent, *: very few, +: few, ++: abundant, +++: very abundant

According to Table 5, *L. closterocerca* in the first, third, and fourth sampling, *L. hamata* in the second and last sampling, *L. pyriformis* in the third sampling were abundant. *P. aduncus* from Cladocera was very abundant in the first, second, and third samplings, while it was abundant in the last sampling.

While *D. longuioides* from Copepoda was very abundant in the third sampling, they were recorded as abundant in the first and last sampling. *Eucyclops serrulatus* was abundant in the second and third sampling; *M. viridi*s was recorded as very abundant in the third sampling and abundant in the second and fourth sampling. *M. mehmetadami* was found very abundant in the fourth sampling and abundant in the other sampling. *P. chiltoni* was abundant in all sampling, *T. dybowski* was very abundant only in the third sampling, *T. prasinus* was very abundant in the second sampling, while it was abundant in the third and fourth sampling. *Elaphoidella* sp. in the fourth sample, and K. xanthi in the second sample were abundant. The least common species *C. gibba, L. bulla, Microcyclops* sp., and *P. viguieri* were detected at only one sampling time and very few (*).

**Discussion**

Zooplankton samples were collected from 29 water wells built for various purposes (to irrigate the flowers on the graves, to use in the construction of graves, and to irrigate the trees in the entire cemetery) in the cemetery complex of Kilis province.

The depth, width, and water depth of the water wells were measured to be between 5 and 17 meters, 1.10 and 2.20 meters, and 2 and 7 meters, respectively.

Groundwater fauna can be used to examine groundwater from an ecological standpoint. Organic matter and dissolved oxygen imported from the surface are essential for faunal biocenoses, such as the living activities of animals in groundwater habitats. Both are made available as a result of groundwater and surface water exchange activities. Hydrological exchange, as well as site variety, must be taken into account while forming communities of unpolluted groundwater. Because they purify the groundwater, groundwater fauna plays a vital role in groundwater ecosystems. Groundwater fauna may also serve as bioindicators, integrating short-, mid-, and long-term changes in environmental conditions within an ecosystem (Malard et al., 1996; Mösslacher, 2000).

The main water sources of water wells are groundwater, rainwater, and leachate water enters the wells with open mouths, making an additional contribution to the existing water. Therefore, the access of planktonic organisms to well water is mainly dependent on groundwater and can then be said to be supported by rainwater. Although zooplankton species are poor in terms of species richness and abundance in groundwater, especially copepods constitute an important community in these waters (Galassi, 2001). With more than 900 species/subspecies known from continental groundwaters, stygobiont copepods inhabit all kinds of aquifers (karstic, fissured, porous), as well as surface/subsurface ecotones (land/water and water/water). As can be seen from the results of our study, copepod species seem to be richer in species diversity than other zooplankton groups.

In this study, a total of 27 species including 12 rotifer species, 1 cladoceran species and 14 copepod species were identified. While the distribution of zooplankton in lakes and streams is generally in the form of Rotifera, Cladocera, and Copepoda, the study of zooplankton biodiversity in groundwater found Copepoda to be the most represented species, followed by Rotifera and Cladocera. So far, two studies have been conducted on zooplankton related to groundwater and water wells in Turkey (Bozkurt, 2019; Bozkurt & Bozça, 2019). In both studies, it was reported that most species were rotifers, followed by copepods and cladocerans. In our study, unlike other studies, copepod species diversity was found to be higher.

The species detected in the study, *C. forficula, C. gibba, C. uncinata, Lepadella patella, Lecane bulla, L. closterocerca, L. flexilis, L. hamata, L. inermis, L. ludwigi, L. pyriformis, L. tenuiseta, P. aduncus, D. languidoides, E. serrulatus, M. viridis, P. chiltoni, T. dybowski, T. prasinus, A. crassa, N. stammeri,* and *P. viguieri* have been reported by various researchers to be cosmopolitan and have a wide distribution in different abundances at different times of the year (Ruttner-Kolisko, 1974; Koste & Shiel, 1987; De Smet, 1996; De Manuel Barrabin, 2000; Stoch & Pospisil, 2000; Ramdani et al., 2001; Rybak & Bledzki, 2010). In addition to all these, they are very tolerant of changes in water quality parameters (Berzins & Pejler, 1987; Koste & Shiel, 1989; Manuel Barrabin, 2000).

It was reported that *M. mehmetadami* (Karaytug et al., 2018) and *K. xanthi* (Bruno & Cottarelli, 2015) were identified first of all from the hyporheic fauna by Karaytug et al. (2018) and Bruno & Cottarelli (2015) respectively. For this reason, the ecological and habitat characteristics of the newly discovered species, which were thought to be hyporheic due to their habitat characteristics, have not been determined yet.

Species of *Microcyclops* and *Elaphoidella* genera found in the study could not be identified due to insufficient samples. However, it is known that some species belonging to these genera are common in groundwater. In addition, the genus *Ectinosoma* is reported for the first time from inland waters of Turkey and the species has not yet been identified.

Some rotifer species in the study, *C. forficula, C. gibba, Colurella uncinata, L. bulla, L. hamata, L. flexilis, L. ludwigi, L. pyriformis,* and *L. tenuiseta* are littoral periphytic rotifers and they mostly live on plant substrata (de Manuel Barrabin, 2000; Hingley, 1993), in the standing and running waters (Koste, 1978; Segers, 1995; Kuczynska-Kippen, 2000). On the other hand, they are occasionally found in the plankton (Braioni & Gelmini, 1983).

Some other rotifer species, *C. gibba, L. closterocerca,* and *L. inermis* are the most common benthic rotifers, but it was frequently observed in plankton samples (Ruttner-Kolisko, 1974). Although *L. closterocerca* mostly prefers temporary ponds, it can also be found in streams (de Manuel Barrabin, 2000). In addition, some of the species in the study, (*L. closterocerca, L. inermis, L. pyriformis, Lepadella patella*), tolerate a wide range of salinity (De Smet, 1996; Walsh et al., 2008). It is reported that the rotifer species included in the study are most common in pH values

between 6.5 and 8.2 and temperature values between 7.8 and 24 degrees (Nogrady & Pourriot, 1995; De Smet, 1996; Koste & Shiel, 1990; De Manuel Barrabin, 2000).

Researchers have reported that *C. forficula* is a free-floating, tube-dwelling species (Dodson, 1984), *C. uncinata* can tolerate a wide variety of mineralization, and *L. bulla* has been associated with interconnected flowing spring pools (Segers, 1995). Further, the limnobiological correlation between physicochemical parameters and rotifer associations revealed, *L. bulla, L. closterocerca, L. hamata* and *L. ludwigi*, as euryokous species, showing tolerance to a wide range of abiotic factors and habitats (Segers, 1995).

*Pleuroxus aduncus*, the only member of Cladocera in the study, is a macrophyte-sediment-related taxon and lives at the bottom and in macrophyte beds. *P. aduncus* is known for a variety of water types, including temporary localities and slightly saline waters, and is the inhabitant of eutrophic waters (salinity up to 2.9%0) (Timms, 1973; Vadadi-Fülöp et al., 2008).

Some copepod species in the study, *D. languidoides, E. serrulatus, M. viridis, T. prasinus, P. chiltoni, T. prasinus, A. crassa, N. stammeri,* and *P. viguieri* are reported by various researchers to live in a wide range of habitats such as caves, wells, groundwater systems, spring waters, ponds, rivers, backwaters, benthic zone of lakes, marshes and swamps (Morton & Bayly, 1977; Pesce & Maggi, 1981; Berzins & Bertilsson, 1990; Lehman & Reid, 1992; Karaytuğ, 1999; Dussart & Defaye, 2006; Lee & Chang, 2007; Tang & Knott, 2008; Galassi et al., 2011; Iepure et al., 2014; Bruno & Cottarelli, 2015; Iepure et al., 2016; Bozkurt, 2017). *T. dybowskii*, one of the copepod species recorded in the study, which was not reported from groundwater and wells, is in perennial ponds, coastal waters (occasional), pelagic zone of ponds and lakes, lives in small water bodies (Maier, 1990). It has also been reported by various researchers that *E. serrulatus* and *N. stammeri* are the most representative taxa in the wells (Iepure et al., 2016).

Some zooplankton species [*Ascomorpha ovalis* (Bergendahl, 1892), *Cephalodella catellina* (Müller, 1786), *C. gibba, C. ventripes* (Dixon-Nuttall, 1901), *Colurella adriatica* Ehrenberg, 1831, *C. colurus* (Ehrenberg, 1830), *C. uncinata, Dicranophorus epicharis* Harring & Myers, 1928, *Euchlanis dilatata* Ehrenberg, 1832, *Filinia longiseta* (Ehrenberg, 1834), *Heterolepadella ehrenbergi* (Perty, 1850), *Keratella cochlearis* (Gosse, 1851), *K. quadrata* (Müller, 1786), *K. tecta* (Gosse, 1851), *K. tropica* (Apstein, 1907), *Lecane bulla, L. closterocerca, L. flexilis, L. hamata, L. lunaris* (Ehrenberg, 1832), *L. pumila* (Rousselet, 1906), *L. tenuiseta, Lepadella acuminata* (Ehrenberg, 1834), *L. patella, Lophocharis salpina* (Ehrenberg, 1834), *Mytilina unguipes* (Lucks, 1912), *Platyias quadricornis* (Ehrenberg, 1832), *Synchaeta stylata* Wierzejski, 1893, *Testudinella elliptica* (Ehrenberg, 1834), *T. patina* (Hermann, 1783), *Trichocerca similis (*Wierzejski, 1893), *T. tigris* (Müller, 1786), *Trichotria tetractis* (Ehrenberg, 1830); *Alona guttata* Sars, 1862, *Bosmina longirostris* (Müller, 1785), *Ceriodaphnia pulchella* Sars, 1862, *C. reticulata* (Jurine, 1820), *Chydorus sphaericus* (Müller 1776), *Diaphanosoma birgei* Korinek, 1981, *Leydigia acanthocercoides* (Fischer, 1854), *Pleuroxus aduncus, Simocephalus vetulus* (Müller, 1776), *Acanthocyclops robustus* (Sars, 1863), *Attheyella crassa, Bryocamptus minutus* (Claus, 1863), *B.*

*zschokkei* (Schmeil, 1893), *Canthocamptus microstaphylinus* Wolf 1905, *Cyclops vicinus* Uljanin, 1875, *Diacyclops bisetosus* (Rehberg, 1880), *D. bicuspidatus* (Claus, 1857), *D. languidus* (Sars, 1863), *Eudiaptomus drieschi* (Poppe and Mrazek, 1895), *Macrocyclops albidus* (Jurine, 1820), *Megacyclops viridis, Nitocra hibernica* (Brady, 1880), *Nitocrella kosswigi* Noodt, 1954, *Paracyclops fimbriatus* (Fischer, 1853), *Speocyclops* sp., *Tropocyclops prasinus*] were previously reported from the water wells of Kuyubeli Village and Yayladağı District (Bozkurt, 2019; Bozkurt & Bozça, 2019). Species reported from other two water well studies and thought to have high groundwater adaptation potential include *C. gibba, C. uncinata, L. bulla, L. closterocerca, L. flexilis, L. hamata, L. tenuiseta, Lepadella patella, P. aduncus, M. viridis, T. prasinus* and *A. crassa* were recorded in this study.

As a result, the fact that most of the rotifer species reported in our study have only been recorded in small numbers in well waters in our country suggests that they can be recorded in a variety of settings. The fact that the copepod species are different in the well studies in all three regions in Turkey supports the idea that the groundwater has a greater diversity of copepod species, as documented in many studies. As a result, the ability of the zooplankton species studied to adjust to environmental conditions and their ecological valence can be stated to be high. Although their habitats are not groundwater, the species detected in our study's water well samples are thought to have infiltrated these wells via zooplankton dispersal processes (winds, water particles, birds, and insects).

**Conflict of Interest**

The author declares that no competing interests.

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# First Report on the Elemental Composition of the Bigeye Thresher Shark *Alopias superciliosus* Lowe, 1841 from the Mediterranean Sea

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## Abstract

Cartilaginous fish species have ecological importance. Besides, the ecotoxicological studies on these species are pretty insufficient. In this study, Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels were determined in muscle, liver, gill, kidney, spleen, stomach, and gonad tissues of *Alopias superciliosus* (Female, 240 cm TL) caught from Mersin Bay. Tissue metal analysis was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A statistical difference was found among the tissues in terms of the metals. Fe was determined to be the highest level in all tissues ($p<0.05$). The relation between tissues in terms of Fe level was determined as Liver>Gill>Spleen>Gonad>Kidney>Stomach>Muscle. Zn was detected at higher levels in the liver and stomach and As in other tissues after Fe. Al has the highest level after Zn and As and was mainly found in the gills. The tissue Cu and Zn levels were found in the same order from highest to lowest as Liver>Gonad>Kidney>Spleen≥ Stomach>Gill>Muscle. Sr was higher in the stomach, gonad, and kidney than in the other tissues. Cd levels were found in higher than Pb levels in the examined tissues. Liver Cd level was determined as 57.37 µg $g^{-1}$ dw. Except for the liver, Mn levels were found low than Cr levels in the examined tissues. The distinction between the tissue levels of the investigated elements has changed depending on the functional differences between the tissues and metal metabolisms.

**Keywords:**

*Alopias superciliosus, elemental composition, tissue level, Mersin Bay*

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## Introduction

Studies examining the accumulation and toxic effects of metals that participate in the marine ecosystem under the influence of natural and anthropogenic sources in marine organisms have

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proven that metals taken through water and food are easily transported in the food chain. Accumulation varies according to species, organization level, habitat, sex, texture, and metal is known (Jardine et al., 2013). The continuity of the participation of metals in nature and their long half-life of them cause vertical and horizontal transfer of them in aquatic ecosystems and progress from the shore to the open waters. In addition, the density of vegetation and the amount of dissolved organic matter in the environment can change the concentration of metals in the aquatic environment.

Cartilaginous fish with a long lifespan can accumulate metals at high levels because they are apex predators at the upper trophic level and make long migrations circumglobally (Delshad et al., 2012; Nicolaus et al., 2016; Adel et al., 2017; Lara et al., 2020). Furthermore, due to the widespread use in different industries such as fin, muscle, liver oil, cartilage, and squalene production, it is necessary to determine the metal levels in the tissues of cartilaginous fish.

Diet is the main route of metal uptake and accumulation in cartilaginous fish (Corsolini et al., 2014), and it has been reported that accumulation differs between tissues. The liver, which is a metabolically active tissue, can accumulate metals at higher concentrations than other tissue due to the leading synthesis site of metallothionein, a metal-binding protein, and glutathione, a tripeptide rich in low molecular weight cysteine (Mull et al., 2012).

The species and tissue examined in ecotoxicological studies with cartilaginous fish are minimal. The gills are target organs because they directly interact with the metal in the environment and are essential in terms of reflecting the metal environment concentration. The digestive organs should be examined in terms of reflecting dietary intake, and the kidneys as the most basic route of excretion. Gonads show a high affinity for accumulation during the long maturation phase in cartilaginous fish. This is important for reproductive success.

A study conducted with *Galeocerdo cuvier* determined that Cu, Zn, and Hg levels vary depending on the growth rate and tissue (Endo et al., 2008). In a study, Cu, Zn, Cd, Hg, and Pb levels were investigated in the fin and muscle tissues of four Elasmobranch species; and it was stated that heavy metal levels varied according to species, metal, and tissue (Ong & Gan, 2017).

*A. superciliosus,* known as the big-eyed thresher shark, is a pelagic oceanodromous species systematically belonging to the Alopiidae family of the Lamniformes order (Riede 2004). It shows a circumglobal distribution in tropical and temperate waters at a depth of 0-730 m (McMillan et al., 2011) and generally 0-100 m (Compagno, 2001). It is located in the pelagic and benthopelagic zones on the continental shelf. It feeds on lancetfish, herring, mackerel, small fish in the pelagic zone, and bottom fish such as European hake and squid in the benthic area.

The distribution of *A. superciliosus* in the Mediterranean is quite limited. Although there are restricted ecotoxicological studies with *A. vulpinus* and *A. pelagicus,* there are no

ecotoxicological studies related to *A. superciliosus*. It is important to be toxicological reports in species with ecological and economic importance. Therefore, it was aimed to determine the Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels in the liver, gill, muscle, kidney, spleen, stomach and gonad of a female *A. superciliosus* specimen caught from Mersin Bay in the present study.

**Materials and Methods**

*Alopias superciliosus*, which was used as a material in the research, was caught incidentally from the Mersin Bay Taşucu coast from 25 m with a trammel net in January 2020. It was determined that the individual was a young female with a total length of 240 cm. The individual was brought to the Museum of Marine Life, Mersin University, and recorded with the catalog number (MEUFC-20-11-127). The liver, gill, muscle, kidney, spleen, gonad, and stomach tissue were dissected, and three samples were taken from each tissue. Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels were determined in tissues. The coordinate (Coordinate: 36°18'17.6"N, 33°51'41.0"E) and map of the area where the sample was caught are presented in Figure 1.

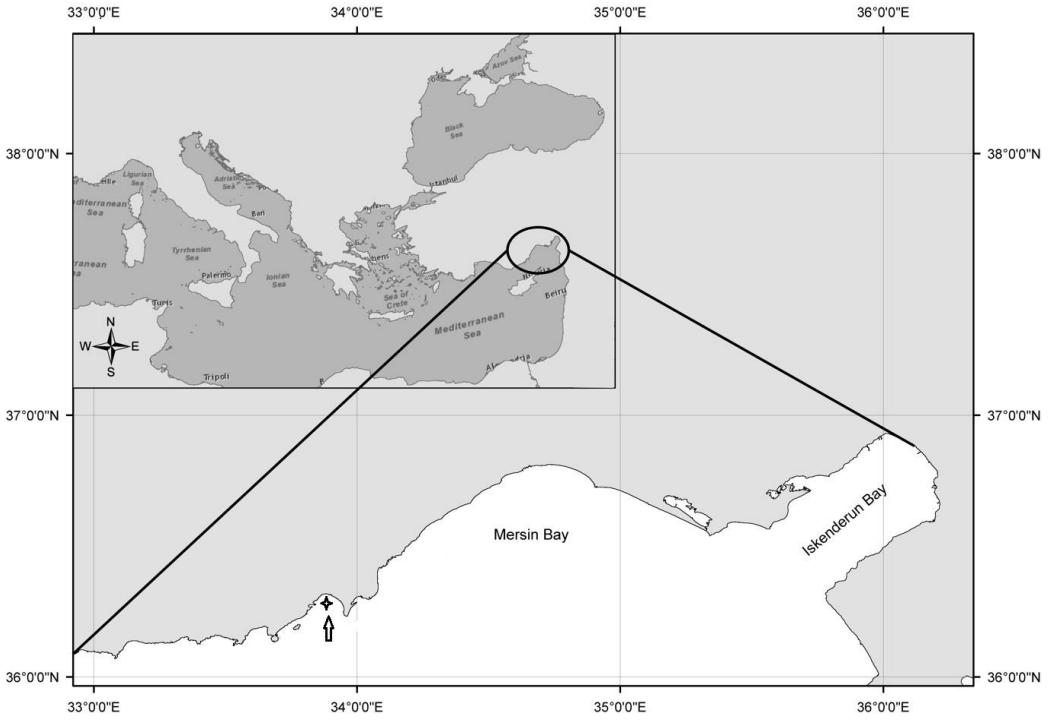


Figure 1. Incidentally captured locality for *Alopias superciliosus* from Taşucu Bay.

The dissected tissues were dried at 95 °C for 72 hours. After the dry weight of the tissues was determined, 2 ml of nitric acid was added (HNO3, 65%, Merck) and digested at 120 °C for 4 hours. The acid-digested samples were then transferred to falcon tubes and filled with 10 ml of bidistilled water (Çiftçi et al., 2021). Finally, samples were analyzed with an Inductively-Coupled

Plasma Mass Spectrometer (ICP-MS, Agilent, 7500ce Model, Japan). ICP-MS operating conditions were the following: radio frequency (RF) (W), 1500; plasma gas flow rate (L $min^{-1}$),15; auxiliary gas flow rate (L $min^{-1}$), 1; carrier gas flow rate (L $min^{-1}$), 1.1; spray chamber T (°C), 2; sample depth (mm), 8.6; sample introduction flow rate (ml $min^{-1}$), 1; nebulizer pump (rps), 0.1; extract lens (V), 1.5. Metals in samples were detected as µg metal g−1 dry weight. High Purity Multi-Standard (Charleston, SC 29423) was used to determine the metal analyses. Standard solutions for calibration curves were prepared by dilutions of the trace elements and potentially toxic metals. International Atomic Energy Agency (IAEA-436) reference material was used to follow the quality of the analytical process. IAEA-436 was analyzed for all elements. The certified value and observed value of the IAEA-436 reference material were compared. Replicate analysis of this reference material showed good accuracy (Table 1).

Table 1. The certificated value and the observed value of reference material IAEA-436.

| Analyte | Certified value | 95% Confidence interval | Observed value |
|---|---|---|---|
| **Al** | 3.06 ± 0.42 | 2.68–3.44 | 3.419 ± 0.11 |
| **Cr** | 0.194 ± 0.058 | 0.168–0.219 | 0.179 ± 0.01 |
| **Mn** | 0.238 ± 0.042 | 0.218–0.257 | 0.238 ± 0.005 |
| **Fe** | 89.3 ± 4.2 | 87.8–90.9 | 99.04 ± 0.826 |
| **Cu** | 1.73 ± 0.19 | 1.66–1.79 | 1.827 ± 0.092 |
| **Zn** | 19.0 ± 1.3 | 18.6–19.4 | 19.876 ± 0.072 |
| **As** | 1.98 ± 0.17 | 1.91–2.06 | 2.19 ± 0.035 |
| **Cd** | 0.052 ± 0.007 | 0.050-0.054 | 0.049 ± 0.021 |
| **Sr** | 0.564 ± 0.062 | 0.523–0.606 | 0.598 ± 0.026 |

The statistical analyses of data were performed using IBM 22 SPSS package program. The metal levels in tissues of *A. superciliosus* were carried out using variance analysis (ANOVA), and Duncan's Multiple Range tests were used to compare the distinction between each group.

**Results**

Al, Cr, Mn, Fe, Cu, Zn, As, Pb, Cd, and Sr levels in the liver, gill, muscle, kidney, spleen, stomach, and gonad tissues of *A. superciliosus* are shown in Table 2. There were statistically significant differences between metals in terms of tissue levels ($p<0.05$).

Fe was found at the highest and Cr at the lowest level in all examined tissues. The tissue Fe level was determined as Liver>Gill>Spleen>Gonad>Kidney>Stomach>Muscle, respectively. Zn in the liver and stomach, As in other tissues, was found to be second place as a higher element after Fe. The tissues were listed as liver>gonad>spleen>gill> kidney>stomach>muscle in order to As level. Al was followed by Zn and As, and the highest level was found in the gill. The tissue Cu and Zn levels were found in the same order from highest to lowest as Liver>Gonad>Kidney> Spleen≥Stomach>Gill>Muscle. Sr was the highest in the stomach, followed by the gonad and kidney. Cd was found at higher levels than Pb in the examined tissues. Liver Cd level was determined as 57.37 μg $g^{-1}$ d.w. Mn was found in low levels after Cr in tissues except for the liver.

Table 2. Metal levels (μg $g^{-1}$ dw) in tissues of *A. superciliosus*

| | **Spleen** $\overline{x} \pm s_x$ | **Stomach** $\overline{x} \pm s_x$ | **Gonad** $\overline{x} \pm s_x$ | **Kidney** $\overline{x} \pm s_x$ | **Gill** $\overline{x} \pm s_x$ | **Liver** $\overline{x} \pm s_x$ | **Muscle** $\overline{x} \pm s_x$ |
|---|---|---|---|---|---|---|---|
| **Al** | 6.26±1.72$^{a}$ | 6.96±1.05$^{a}$ | 5.84±0.68$^{a}$ | 6.95±1.65$^{a}$ | 56.65±9.08$^{b}$ | 4.80±0.27$^{a}$ | 5.67±1.49$^{a}$ |
| **Cr** | 0.39±0.06$^{ab}$ | 0.15±0.05$^{a}$ | 0.40±0.04$^{ab}$ | 0.26±0.02$^{a}$ | 0.85±0.15$^{c}$ | 0.54±0.06$^{b}$ | 0.31±0.10$^{ab}$ |
| **Mn** | 0.57±0.05$^{a}$ | 1.79±0.22$^{a}$ | 1.61±0.30$^{a}$ | 1.87±0.51$^{a}$ | 1.52±0.22$^{a}$ | 5.00±0.83$^{b}$ | 1.00±0.15$^{a}$ |
| **Fe** | 676.94±110.71$^{c}$ | 74.23±4.93$^{a}$ | 478.31±88.82$^{bc}$ | 316.55±96.26$^{b}$ | 1300.01±96.14$^{d}$ | 1831.3±48.62$^{e}$ | 48.27±7.03$^{a}$ |
| **Cu** | 3.59±0.51$^{a}$ | 5.81±0.62$^{a}$ | 20.66±6.78$^{b}$ | 10.37±1.02$^{ab}$ | 2.71±0.41$^{a}$ | 142.41±9.37$^{b}$ | 2.32±0.57$^{a}$ |
| **Zn** | 50.51±6.52$^{ab}$ | 49.05±0.58$^{ab}$ | 92.53±14.20$^{bc}$ | 75.14±16.47$^{bc}$ | 42.50±3.78$^{ab}$ | 309.66±18.87$^{c}$ | 25.70±2.99$^{a}$ |
| **As** | 141.58±16.37$^{b}$ | 47.10±5.60$^{a}$ | 213.74±19.29$^{c}$ | 104.26±8.12$^{b}$ | 124.95±12.43$^{b}$ | 243.85±22.32$^{c}$ | 42.81±2.47$^{a}$ |
| **Pb** | 2.98±0.63$^{a}$ | 3.02±0.61$^{a}$ | 3.46±0.21$^{a}$ | 3.68±0.21$^{a}$ | 3.85±0.52$^{a}$ | 2.84±0.04$^{a}$ | 3.56±0.86$^{a}$ |
| **Cd** | 2.93±0.37$^{ab}$ | 2.89±0.18$^{ab}$ | 9.13±2.21$^{b}$ | 5.98±0.92$^{ab}$ | 1.96±0.21$^{ab}$ | 57.37±5.24$^{c}$ | 0.97±0.17$^{a}$ |
| **Sr** | 5.82±0.50$^{a}$ | 19.11±3.21$^{b}$ | 15.71±1.22$^{b}$ | 15.63±4.2$^{b}$ | 8.85±1.11$^{a}$ | 5.95±0.37$^{a}$ | 4.01±0.42$^{a}$ |

*The different letters (a, b, c, d) in each row indicate the statistical differences ($p<0.05$) between tissues
$\overline{x} \pm s_x$: Mean±standard error

## Discussion

Increasing concentrations of essential elements such as Fe, Cu, and Zn, which are vital for animals at a certain concentration, and deficient concentrations of non-essential elements such as Cd and Pb, which are not biologically required, cause accumulation and toxicity in tissues. In the study, the gill, liver, spleen, muscle, kidney, and gonad tissue levels of essential (Fe, Cu, Zn, Mn, Cr, Sr) and non-essential (Al, As, Pb, and Cd) elements of *A. superciliosus* were determined. Among the essential elements, Fe was found at the highest level. Tissue Fe levels of the species were determined as Liver>Gill>Spleen>Gonad>Kidney> Stomach>Muscle, respectively ($p<0.05$).

Fe is responsible for many biochemical reactions, takes part in oxygen transport by participating in the structure of hemoglobin and myoglobin, and takes part in the electron transport system by participating in the structure of cytochromes in animals. In animals, 60% of the total Fe is found in blood hemoglobin, 3-7% in myoglobin in muscle tissue, while the rest is in the liver, spleen, kidney, bone marrow, and muscles. The excretion of Fe, whose absorption in the body is

very low, is controlled by Fe homeostasis. The Fe level in animals varies depending on the species, developmental stage, sex, feeding preference, and disease. Muscle Fe levels in *Prionace glauca*, *Carcharhinus falciformis*, and *A. pelagicus* sampled from the Mexico coast were determined as 445.3±673.63, 420.0±393.22, 396.3±306.50 mg $kg^{-1}$ ww, respectively, and muscle Fe levels were found to be higher (41-61%) in females compared to males (Álvaro-Berlanga et al., 2021). In the present study, the muscle Fe level of *A. superciliosus* was determined as 48.27±7.03 μg $g^{-1}$ dw. Fe was found at the highest level in the liver of *A. superciliosus* among the other tissues. The liver is the most important iron reserve area. The gills have the highest Fe levels after the liver (Doğdu et al., 2021). The wide capillary network that provides gas exchange during respiration explains the high level of Fe in the gills. Spleen is a hematopoietic tissue and Fe level was found to be high in the third place. Fe, together with Cu, is an essential element responsible for reproduction. "Sertoli and Leydig cells" in the male reproductive system are important sources of ferritin and serve as a ready source of Fe for developing spermatozoa as well as protecting testicular tissue (Toebosch et al., 1987; Wise et al., 2003). Fe level in *A. superciliosus* gonad tissue may indicate that the individual is either in the reproductive period to reach reproductive or maturity. It is known that the known reproductive maturity range of the species is 154-341 cm, with an average of 253 cm (Compagno 1984). The kidneys are responsible for homeostasis and excretion in fish (Goldstein & Schnellmann 1996; Wendelaar Bonga & Lock 2008). Since cartilaginous fish are hyperosmotic, freshwater and marine bony fish show kidney functions together. In this study, kidney tissue Fe level can be explained by homeostatic control. Heme iron is the most important source of Fe in top predator species with long lifespans, such as sharks. The low pH of the stomach allows Fe to be converted to the ionic form during digestion, and cellular absorption of iron in the ionic form is faster. This may explain why the Fe level in *A. superciliosus* stomach tissue is higher than the muscle tissue level.

Cu and Zn are trace elements that function as cofactors by participating in the structure of many enzymes in animal organisms, and they cause disturbances in metabolic and physiological events over a certain concentration range (Michalska-Mosiej et al., 2016). Zn participates in the structure of more enzymes than Cu, so it is generally found at higher levels than Cu in Teleost and Chondrichthyes fish species (Mendil et al., 2010; Olgunoğlu et al., 2015; Raimundo et al., 2015; Álvaro-Berlanga et al., 2021). Adel et al. (2017) reported that the muscle Cu level *in Carcharhinus dussumieri* was higher than the Zn, which could be explained by the antagonistic effect between metals. Cu and Zn levels in tissues of *A. superciliosus* were determined as Liver>Gonad>Kidney >Spleen>Stomach>Gill>Muscle, respectively. The proportional similarity in the tissue levels of both elements can be explained by the functional role of the tissues and metal metabolism.

The arsenic originating from the earth's crust is highly involved in the aquatic ecosystem with anthropogenic sources (Kumari et al., 2017). Arsenic is a nonmetallic element found in different forms in biological systems. The presence of organic arsenic compounds in fish and other aquatic fauna and flora has been reported in many studies (Francesconi et al., 1994; Schmeisser et

al., 2004; Soeroes et al., 2005; Grotti et al., 2008; Rahman & Hasegawa 2012). It is known that arsenic, which is in the inorganic form in water, turns into a harmless form as a result of methylation with aquatic flora and is stored in the muscle and liver as organic compounds such as arsenobetaine and arsenolipid in biota (Duker et al., 2005; Bears et al., 2006). The liver has an important role in the biotransformation of inorganic arsenic and is stored in the liver in various bony fish species (Cockell et al., 1991; Ohki et al., 2002). However, it has been reported that arsenic is found at a higher level in muscle tissue than liver in some teleost fish (C˘elechovska´ et al., 2011; Tyokumbur et al., 2014; Çiftçi et al., 2021). Arsenic was found at the highest level in the liver and gonad tissues of *A. superciliosus* and lowest in muscle tissue in the present study. The accumulation of high biotransformation ability elements, such as arsenic, in tissues may vary depending on the species, life span, organization level, nutritional preference, and habitat. In this study, the gill and kidney tissue As levels were found to be higher than the muscle tissue, which may indicate that excretion is high.

The gills are one of the main uptake routes of metals in the aquatic environment by fish, and especially the uptake and transport of +2 valence elements in the body occur at a higher level. Aluminum is added to aquatic ecosystems mostly with the effect of anthropogenic factors. Its main uptake by fish takes place through gills, which are in direct interaction with the environment. Aluminum has a valence of +3. This situation weakens the competition of aluminum with the +2 valence elements that can be easily taken from the Ca channels in the gills (Rosseland et al., 1990; Exley et al., 1991; Monette et al., 2008). Its low water solubility is another factor limiting its accumulation. Acidic environments increase Al solubility and cause toxic effects in aquatic organisms. Wauer and Teien (2010) stated that gill tissue is a bioindicator for Al accumulation in fish. Al was found in the highest concentration in the gill tissue of *A. superciliosus* ($p<0.05$), and there was no statistical difference in other tissues ($p>0.05$). The most important reason for this may be the limited transport of aluminum between tissues as a result of binding to functional groups located both apically and within the lamellar epithelial cells on the gill surface (Exley et al., 1991). Muscle tissue Al level was reported as 0.83 μg $g^{-1}$ dw (Marques et al., 2021) in *Scylorhinus canicula* sampled from the Atlantic Ocean and 1.34 mg kg-1 ww in *Mustelus mustelus* sampled from Langebaan Lagoon, South Africa (Bosch et al., 2016). Muscle Al level in *A. superciliosus* was found to be 5.67 μg $g^{-1}$ dw in the present study. Our findings were higher than in previous studies.

In cartilaginous fish, tissue Pb level varies depending on the species. The muscle tissue Pb level was reported as 2.89 in *P. galuca,* 4.08 in *C. falciformis,* and 2.61 mg $kg^{-1}$ ww in *A. pelagicus* (Álvaro-Berlanga et al., 2021). The muscle tissue Pb level of *A. superciliosus* was determined as 3.56 μg $g^{-1}$ dw, and no statistical difference was found between the other tissues in terms of Pb level ($p>0.05$).

It has been determined that liver Cd levels are high in studies conducted with various shark species worldwide. In *P. glauca* sampled from Baja California Sur, Mexico, liver Cd level was

34.66 mg $kg^{-1}$ ww (Barrera-Garcia et al., 2013), in *C. calciformis* 284.55 mg kg-1 ww (Terrazas-Lopez et al., 2016), 86.53 mg $kg^{-1}$ ww in *A. pelagicus* (Lara et al., 2020) and 19.77 mg $kg^{-1}$ ww in *Sphyrna zygaena* sampled from the Mediterranean (Storelli et al., 2003). In this study, the liver Cd level of *A. supercilosus* sampled from the Northeast Mediterranean was found to be 57.37 μg $g^{-1}$ dw. High liver Cd levels in sharks may be related to their longevity, being top predators, and feeding preferences. One of the possible reasons for the high level of Cd in the liver is the synthesis of metallothionein and glutathione, which are low molecular weight, rich in cysteine, and function in metal binding, mainly in the liver.

Due to the functional properties of the liver, they can accumulate biologically required metals at high concentrations. Especially at the beginning of the developmental stage, Cu and Zn levels in the liver are quite high (Mull et al., 2012; Corsolini et al., 2014). Among these essential elements, Zn, besides its basic functions, has an important role in reducing Cd toxicity with its capacity to activate metal transcription factor (MTF-1), which stimulates metallothionein synthesis (Di Giulio & Meyer, 2008; Hahn & Hestermann, 2008). Barrera-García et al. (2013) emphasized that liver Zn level may be related to Cd level in *P. glauca*, for the reasons specified. The high level of *A. superciliosus* liver Zn and Cd in this study may also be due to the same mechanism. In this study, Cd was found the second-highest level in the gonad after the liver. Metals are actively transported to the gonad tissue during the reproductive period. In this study, the high level of Cd accumulation in the gonad tissue by *A. superciliosus* can be explained by the tendency to accumulate Cd at high levels in the gonad tissue due to its similarity to Ca to reach the individual's reproductive maturity.

Strontium was found at higher levels in the stomach, gonads, and kidneys of *A. superciliosus* than in other tissues in the present study. It is possible that this element, which is responsible for the mineralization of bone and cartilage tissue and invertebrate shells, may have been taken by food.

Cr and Mn were found at the lowest level among the metals examined in this study. *Rhincodon typus* has been reported as Cr 5.21 μg $g^{-1}$ ww and Mn 4.45 μg $g^{-1}$ ww in muscle tissue (Pancaldi et al., 2021). Muscle Cr and Mn levels in *A. superciliosus* were found to be lower than in previous studies. This may be related to the concentration of these metals in the environment, their ranks in the food chain, or their metabolism in the organism.

In conclusion, sharks are predators at the upper trophic level of the food chain and have a very long lifespan. The long periods of growth and reproductive maturity cause chronic accumulation of metals added to the environment through different sources in these species. The level of accumulation of metals taken into the body, mainly through food and water, cannot be adequately explained by regional pollution for species that make long migrations between oceans and seas, such as sharks. While migratory species may accumulate in certain concentrations in polluted areas, they may decrease tissue metal levels as a result of natural detoxification and depuration in areas far from metal effects. Therefore, tissue metal levels can be interpreted with

nutritional preferences. In sharks that feed on benthic, benthopelagic fish, and invertebrate species, it is possible that the accumulation may be acquired mostly through contaminated prey. It is very important to monitor tissue metal concentrations in order to protect human health in these species, which are widely used in different industrial areas. *A. superciliosus* is a species with a limited number of individuals in the Mediterranean. The data obtained in this study is important in forming the species' first ecotoxicological report.

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**Conflict of Interest**

The authors declare that they have no competing interests.

**Author Contributions**

N.Ç. wrote the main manuscript, DA: Sampled the species and designed the research, BC: prepared table and figure.

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# The Genotoxic Damage in *Cyprinus carpio* Exposed to Abamectin

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## Abstract

The pesticide abamectin, which is often used in agriculture, poses a threat to aquatic animals. Though its toxicity to fish has not yet been fully understood. In this study, we used the comet assay to examine the effects of being subjected to various dosages of abamectin on the genotoxic impact of abamectin in *Cyprinus carpio*. During 10 days, common carp were exposed to three different doses of abamectin (0.3, 0.6, and 0.9 mg $L^{-1}$) based on previously discovered levels in aquatic environments. Toward the completion of the investigation, the Comet assay was used to assess the damage frequency (%), Arbitrary unit (%), and Genetic damage index (%) in carp gill and liver cells. The greatest damage frequencies of % 74.333±0.577 and % 70.333±2.082 were significantly found in the 0.9 mg $L^{-1}$ group in the gill and liver cells, respectively ($P<0.001$). Our results showed a considerable increase in DNA strand breaks in *C. carpio* after exposure to abamectin, suggesting the pesticide's capacity to be genotoxic to fish.

**Keywords:**

*Abamectin, DNA damage, pesticide, comet assay*

**Article history:**

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## Introduction

The usage of pesticides in agriculture has increased the amount of food produced worldwide (Alexandratos & Bruinsma, 2012; Santos et al., 2023). Yet, as more and more pesticides are employed to boost agricultural production, the situation is growing more dangerous. Thus, there are concerns over the possibility of environmental pollution and its consequences on creatures other than the targets (Blahova et al., 2020). By faulty container handling, transportation problems, and direct application, pesticides can pollute land and water. The mechanisms of retention, transformation, and transportation all have a role in how pesticides behave in the environment (Carvalho, 2017). Pesticides can have a variety of biological impacts and can affect several

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biochemical processes that are universal to all species. (Turan & Ergenler, 2022; Tresnakova et al., 2022).

Additional environmental factors and pesticides can produce oxidative stress, which is caused by a balance between oxidative and anti-oxidative stress mechanisms at the cellular level. Antioxidant enzymes and non-enzymatic antioxidants function to absorb reactive oxygen species (ROS) and protect cells from oxidative stress damage (Liang et al., 2017; Ergenler & Turan, 2022). In addition, oxidative stress will increase the inflammatory response and affect how the cells respond to infections and toxic chemicals as sources of harmful aggravation.

A group of substances known as "abamectin" is employed for human consumption as a veterinarian insecticide and repellent. (Pitterna et al., 2009; Liu et al., 2020). In 1978, S*treptomyces avermitilis* and fermentative actinomyces were used to isolate the first chemicals in this class. (McCavera et al., 2007; Prichard et al., 2012). Abamectin is a mixture of avermectins (around 80% avermectin $B_{1a}$ and 20% avermectin $B_{1b}$) (Yu et al., 2017) (Figure 1).

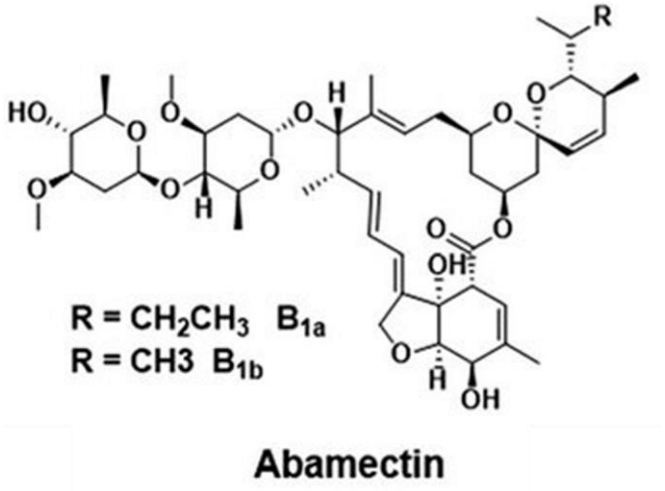


Figure 1. The chemical structure of abamectin (Yu et al., 2017).

Abamectin can accumulate in animal tissues since it is known to be diffuse, slightly soluble in water, and soluble in organic solvents. (Prasse et al., 2009; Lumaret et al., 2012; Santos et al., 2023).The usage of pesticides in aquatic environments and the harmful effects of pesticides on animals have been investigated in several research trials ( Novelli et al., 2012; Alm et al., 2017; Hong et al., 2020; Turan & Ergenler, 2022; Ergenler & Turan, 2022). Less is understood about their harm to aquatic habitats, particularly fish (Turan & Ergenler, 2022; Piriscila et al., 2023; Feng et al., 2023). Using the micronucleus test and comet assay, recent studies by Turan & Ergenler (2022), Ergenler & Turan (2022) revealed the genotoxic properties of acetamipridine and thiamethoxam in a common carp, *Cyprinus carpio*. Pesticides end up in the water when weeds in ecosystems and reservoirs of water are controlled. Since there is an increasing concern over the

presence of genotoxins in the aquatic environment, it is essential to develop methods for recognizing genotoxic chemicals. Additionally, it is unknown if interactions between aquatic organisms and abamectin lead to DNA damage (Feng et al., 2023).

Abamectin, like many other pesticides, can pollute regions outside of its targeted usage and harm creatures that are not their targets. Because of its brief half-life, abamectin is not commonly found in high concentrations in freshwater, but it is nonetheless very poisonous and also can affect aquatic creatures, including many fish species (Bai & Ogburn, 2006).

Fish is one of many aquatic organisms that serve as important biological monitors of aquatic ecosystems. Fish are the largest consumers and contribute significantly to the aquatic food chain by controlling the degree of pollution in aquatic ecosystems. Fish are directly exposed to many xenobiotics, making them ideal indicator organisms for measuring and monitoring water pollution. When foreign substances or carcinogens come into contact with fish, they trigger various interactions between the body's biological and chemical systems, ultimately resulting in biochemical abnormalities. Therefore, it is important to identify the mechanisms of pollution effects and all related countermeasures. To measure the quality of aquatic systems, fish can be used as biomarkers of water pollution (Bonomo et al., 2021). As a result of their capacity to metabolize and absorb contaminants in their systems, fish are among the most appropriate species for potential risk evaluation (Turan & Ergenler, 2019; 2022).

To minimize negative impacts on non-target creatures and public health, it is essential and critical to monitor for harmful effects and screen for various pesticides. Consequently, the purpose of this work was to use the comet test to investigate the genotoxic effects of abamectin in *Cyprinus carpio,* a model fish species.

## Materials and Methods

### *Experimental Design*

A total of 200 common carp (*Cyprinus carpio* Linnaeus, 1758), weighing an average of 2.50 g, were used in the experiment. The carp were acclimated for 15 days in a well-ventilated thirty-litre glass tank filled with dechlorinated water that had constant light periods (12:12 light/dark cycle). 24 hours passed between the last feeding and the time the animals were exposed to the pesticide. The specimens were fed commercial carp feed at a rate of 3% of their body weight. After acclimation, the fish were divided into two groups with n = 15 each: an experiment group and a group that served as a control. Three separate abamectin concentrations (0.3, 0.6, and 0.9 g $L^{-1}$) were chosen to symbolize an acute test lasting a week based on previously observed aquatic environmental values for ten days. There were 45 identical fish in each treatment group. At the end of the experiment, fish were given a dose of five milligrams per liter of quinaldine sulfates (Sigma Chemical Company, Germany) to put them to sleep (Yanar & Genç, 2004). When the specimens

stopped reacting to physical stimuli, they were only handled to extract tissue (liver and gills) for the Comet test (after around 1 to 2 minutes).

### *Comet Assay*

An upgraded version of the Cavalcante et al., (2008) method employing gill cell suspension, cell pellet retention, and single-cell gel electrophoresis was used to conduct the comet experiment. Each slide was examined under a fluorescence microscope Image2M Zeiss at X40 magnification after being stained with ethidium bromide and neutralized with ice-cold 0.4 M Tris solution. Images of 100 cells from each sample were utilized to visually evaluate the nucleoids, which were divided into five groups. For comparison, the damage proportion (% DF), arbitrary values (AU), and DNA damage rating (GDI) were calculated.

### *Statistical Analysis*

Before doing any statistical calculations, the data were checked for normality and homogeneity. A one-way analysis of variance was then performed to see whether there were any significant differences between the treatment groups. The means were analyzed using a one-way ANOVA, and differences were considered statistically meaningful at ($P<0.05$). (Norusis, 1993).

## Results

Table 1 presents averages and standard deviations for gill and liver cells of C. Carpio damage frequencies (% DF), arbitrary unit value (AU), as well as genetic damage index (% GDI).

Table 1. The averages and standard deviations for DNA damage in control and different concentrations of abamectin-treated carp gills and liver. (n=15).

| Groups ($g\ L^{-1}$) | Damage Frequency (%) | Arbitrary Unit AU | Genetic Damage Index (%) |
|---|---|---|---|
| **Liver** | | | |
| Control | $26.000\pm3.472^{a}$ | $49.333\pm8.014^{a}$ | $0.493\pm0.814^{a}$ |
| 0.3 | $61.000\pm1.000^{b}$ | $158.333\pm1.528^{b}$ | $1.583\pm0.152^{b}$ |
| 0.6 | $65.000\pm1.000^{b}$ | $173.667\pm7.572^{c}$ | $1.736\pm0.752^{c}$ |
| 0.9 | $74.333\pm0.577^{c}$ | $181.000\pm3.606^{c}$ | $1.810\pm0.360^{c}$ |
| P | *** | *** | *** |
| **Gill** | | | |
| Control | $24.000\pm0.577^{a}$ | $67.333\pm1.452^{a}$ | $0.673\pm0.145^{a}$ |
| 0.3 | $56.333\pm1.528^{b}$ | $114.667\pm8.145^{b}$ | $1.146\pm0.814^{b}$ |
| 0.6 | $62.333\pm2.309^{c}$ | $159.000\pm8.660^{c}$ | $1.590\pm0.866^{c}$ |
| 0.9 | $70.333\pm2.082^{d}$ | $157.667\pm6.506^{c}$ | $1.577\pm0.650^{c}$ |
| P | *** | *** | *** |

The information is presented as the numeric mean and standard deviation. Significant deviations are indicated by values with various superscripts in each section. Determine the level of significance between the three concentrations of abamectin and DNA damage in the gill tissues of carps taken from the control (*, $P<0.001$).

The damage frequency (%), arbitrary unit values (AU), and genetic damage index (%) in the gill and liver cells of *C. carpio* subjected to the control and three different concentrations of abamectin are reported together with means and standard deviations in Table 1. The DNA damage frequency, AU and GDI parameters are also impacted by abamectin therapy ($P<0.001$). The study's findings showed that the 0.9 mg $L^{-1}$ group substantially had higher damage frequencies (%) at gill and liver cells, correspondingly, of 74.333±0.577 and 70.333±2.082 ($P<0.001$) (Table 1).

The lowest damage frequencies (%) were 24.000±0.577 and 26.000±3.472 discovered in this report's control group's liver and gill cells, accordingly. In addition, it was shown that additional damage markers (AU and GDI) were considerably greater ($P<0.001$) in the gill and liver tissues of the 0.3 and 0.6 mg $L^{-1}$ group compared to the control group (Table 1). In this study, the control group notably had the least AU and GDI. The highest AU and GDI for gill were found to be 181.000±3.606 % and 1.810±0.360 % as a result of the study, while the highest AU and GDI for liver were found to be 157.667±6.506 and 1.577±0.650 % accordingly, in the 0.9 mg $L^{-1}$ group for gill and liver cells ($P<0.001$). In this study, the DNA damage increased due to the increase in the concentrations of abamectin.

**Discussion**

Pesticides have widespread applications and are frequently employed to protect agricultural productivity. Yet, the consequences of the pesticides sprayed on agriculture might not only operate on target pest populations but additionally have effects on non-target species. Aquatic pesticide exposure is thought to come through runoff, leaching, spray drift, preferred migration, and via soil high porosity, or a mixture of such activities through agricultural regions. Pesticides are mostly transferred to aquatic environments via discharge, although the rate of movement is influenced either by different soil types, the physicochemical characteristics of the pesticides, the time and application rate, and the precipitation following pesticide application. Degradation of pesticides or adsorption to surfaces can be caused by abiotic mechanisms such as photodecomposition by sunshine or breakdown by water (Sumudumali et al., 2021). Yet, due to their indiscriminate and unregulated use, their use might have an impact on species that are not the target. Depending on the type of pesticide, its concentration, the length of exposure, and each one's vulnerability to other variables, the impacts of pesticides might show in different ways and to varying degrees. DNA damage and pesticide exposure have been linked to a wide range of issues. Pesticide exposure has been associated with DNA damage in many living things. Pesticide exposure causes changes in the genetic population, especially DNA damage (Valencia-Quintana et al., 2023).

The comet test may be utilized to assess pesticide exposure. The single-cell gel electrophoresis test has been employed in the evaluation of food items in ecotoxicity, radioactive genetics, ecologic genotoxicity, and genetic toxicology. It has also been used to conduct studies and genetic toxicology brought on by exposure to potentially disruptive chemicals, at the level of

diagnosis, as a result of lifestyle decisions, or as a result of the interaction between diet and anti-oxidant substances use on carcinogenesis.(Liao et al., 2009; Hayat et al., 2018).

There aren't many studies on the toxicity abamectin causes in carp, even though its toxicological effects have been extensively researched. In this investigation, abamectin damage led to DNA damage in the liver and gill material of carp. Many studies have found that creatures exposed to pesticides develop cells with Strand breaks. Below are some studies on creatures that have been introduced to pesticides.

Abamectin damages several tissues and organs in aquatic organisms (Mohammed et al., 2018). In zebrafish investigations, exposure to abamectin damaged the structure and function of the gill tissues, eventually leading to oxygen starvation and mortality (Novelli et al., 2016). According to Feng et al. (2023) the carp gill filament structure was harmed with increasing doses of abamectin exposed, alongside distended and tissue necrosis gill epithelial cells and cells that were inflammatory, suggesting that abamectin hindered the integrity of the carp gill and impacted the carp's respiratory function.

Hepatocytes are being employed for the first time to assess the genotoxicity of Abamectin to *S. prenanti*. Just one day of treatment was necessary for DNA damage to manifest, and OTM levels increased in a dose- and time-dependent way. Even at a density (0.5 g/L) that was significantly lower than that of the environmental level, Abamectin produced substantial Genotoxicity in *S. prenanti* liver hepatocytes (Novelli et al., 2016), in addition to remaining densities 24 hours after implementation in aquatic species (Feng et al., 2023).

The aquatic organisms, such as Daphnia, were quite dangerous to Abamectin; for example, 48-h Abamectin EC50 measurements for *Daphnia similis* and *Daphnia magna* have been identified as 5.1 ng/L and 0.25 g/L, consequently (Novelli et al., 2012). The outcomes for fish were much better; for *Danio rerio*, the 48-h LC50 was 59 g/LV. Fish rates were fairly high. The numbers for fish were significantly higher; for example, the 48-hour LC50 for *Danio rerio* was 59 g/L (Alm et al., 2017). The toxicology of this substance varied depending on the variety; for example, the Abamectin $LC_{50}$ values for the rainbow trout (*Oncorhynchus mykiss*), bluegill fishing sunfish (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), and carp (*Cyprinidae sp*.) were 3.2, 9.6, 24 and 42 g/L, accordingly (Novelli et al., 2012). Based on another study, the $LC_{50}$ values for Abamectin on common carp and *Tilapia mosambica* were 0.475 and 6.828 respectively (Jasmine et al., 2008). Aquatic life was quite harmful to Abamectin. Abamectin was shown to be dangerous and to induce genotoxicity in living things within this inquiry once the gill and liver tissues were investigated. According to our research, abamectin exposure significantly increased the number of DNA breakages in *C. carpio*, indicating that the pesticide may be benign to fish. Our outcomes concur with those of previous studies.

In conclusions, we might recommend utilizing common carp as a model organism for eco-toxicological investigations after analyzing the sensitivity of this agricultural toxin to typical

reference contaminants. Hence, more research using different agricultural pesticides here on species is needed for the purpose of assessing their eligibility for toxicity detection, which is required for water environment monitoring tools.

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**Conflict of Interest**

The authors declare that they have no competing interests.

**Author Contributions**

F.T. performed all the experiments and drafted the main manuscript text. F.T. and A.E. collected samples and performed analysis.

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# Conducting Polymer Films on Zn Deposited Carbon Electrode

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## Abstract

Zn plating on carbon steel (CS) was achieved applying current of 4 mA with galvanostatic technique in acidic medium. Zn particles had homogenous, smooth with spherical structure. It was shown that the Zn particles exhibited active behavior on CS substrate. Poly(aniline), poly(pyrrole), poly(N-methylpyrrole) and poly(o-anisidine) homopolymer films were obtained on CS/Zn electrode. Evaluation of anticorrosion performance of these homopolymer coatings in 3.5 % NaCl solution was investigated by using AC impedance spectroscopy (EIS) technique, anodic polarization and the $E_{ocp}$-time curves. Homopolymer films exhibited significant physical barrier behavior on Zn plated carbon steel, in longer exposure time.

**Keywords:**

*Homopolymer, zinc plating, corrosion*

**Article history:**

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## Introduction

In industry, iron-based metals such as mild and stainless steel have been widely used in numerous applications for many years. The corrosion of the oxidizable metals has become one of the most important problems in the world. Therefore, protective coatings such as organics, silicones, inorganic compounds and metallic coatings have been used widely for metal corrosion control (Guenbour et al., 2000; Kilinççeker et al., 2008; Ozyilmaz, 2011; Kilinççeker et al., 2013). Zinc coatings via electrodeposition technique are considered as one of the many ways of corrosion protection of steel. The use of zinc and zinc-based alloys for improving the corrosion resistance of carbon steel has been growing worldwide and as a coating for high-cost cadmium coating and toxic. Generally metallic zinc based thin film layers provide cathodic protection as sacrificial anode on

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the base metal (Bajat et al., 2001; Martins et al., 2004). In industrial area, metallic surfaces are still coated with thin chromate or phosphate layers, thus improving the corrosion resistance of these metals (Sohi & Jalali, 2003). However, considering that these coatings occur to be applied in parts such as bolts and nuts or in automotive industry, they appear not to last for longer periods. Therefore, when the coatings corrode or scale off, they will leave the base metal vulnerable to corrosion. On the other hand, chromatings and phosphate coatings are not only costly, but they also cause pollution. Thus, scientific studies tend to aim other coating techniques which provide longer protection at lower expense as an alternative to chromatings and phosphate coatings. Therefore, recently conducting polymers are used to enhance corrosion resistance of metallic deposition, which is used in several areas such as automotive accessories, industrial pipes and household items. Today, recent techniques curtain practices such as thin film coatings have a considerable role. Polymeric coatings as monolayer and bilayer offer good protection against oxidizable metal and alloy corrosion. The protection of iron and iron-based metal against corrosion via the use of metallic and polymeric coatings have been the subject of considerable research in many years. The polymeric coatings have some advantages as the ease of their deposition, low toxicity, and low impact on the environment and human health. Several studies related to the use of conducting polymer coatings on metals and alloys for corrosion protection have been reported in recent years. Polyaniline (PANI) and its derivatives are one of the most interesting conducting polymers because of ease in production, environmental stability, antitoxic property and adjustable conductivity. These coatings, which provide a barrier property to oxidizable metals such as copper and mild steel, have already found numerous applications in various industrial sectors including the automotive industry, biosensors, electric and electronic industry (Kim et al., 2000; Shah et al., 2002; Ozyilmaz et al., 2006; Ozyilmaz et al., 2013; Bagherzadeh et al., 2016; Ozyilmaz et al., 2018; Yalçınkaya et al., 2021). Camalet et al. clearly state that the synthesis of polyaniline films on zinc and zinc-nickel alloy in acidic solution such as oxalic acid was unsuccessful (Camalet et al., 1998). However, in our earlier work (Özyılmaz et al., 2005) we reported that polyaniline film which was synthesized in acidic solution such as oxalic acid on nickel (1μm) plated mild steel electrode was successfully used against to corrosion. It was also reported that zinc-cobalt alloy deposited carbon steel (CS/ZnCo) electrode was modified with PANI film in neutral solution as sodium tartrate, in our previous work (Ozyilmaz et al., 2013). It was found that this coating was considerably suitable for the protection of carbon steel.

This study aims to electrochemically synthesize poly(aniline), poly(pyrrole), poly(N-methylpyrrole) and poly(o-anisidine) homoopolymer films on thin zinc (Zn) plated carbon steel in sodium oxalate (NaOX) medium, which would enable the passivation of the Zn plated carbon steel surface. The corrosion performances of Zn plated carbon steel substrates with and without homopolymer coating were investigated in 3.5 % NaCl and compared using the AC impedance diagrams, anodic polarization curves, time-open circuit potential curves and linear sweep voltammograms.

## Materials and Methods

All the chemicals were purchased from Merck. All electrochemical experiments were carried out in a standard one-compartment, three-electrode cell. The reference electrode was Ag/AgCl (3 M, KCl) and the counter electrode was a platinum sheet with surface area of 0.36 $cm^2$. In this study, all electrode potential values were referred to this reference electrode. In this study, the working electrode obtained from Metal At company was carbon steel with the composition: 0.0561 % C, 0.4498 % Mn, 0.0103 % P, 0.0036 % S, 0.14085 Si and 99.3394 % Fe. The surface of this electrode was carefully polished with abrasive paper (1200 grid), degreased with 1/1 acetone/ethanol mixture, washed with distilled water and dried. CHI 606C and CHI 660B model digital electrochemical analyzers were used for all electrochemical measurements. Zn plating was obtained using a bath based on 18.25 zinc chloride ($ZnCl_2$), 65.70 ammonium chloride ($NH_4Cl$), 14.59 carrier (surtec-758-1) and 1.46 polisher (surtec-758-2) by weight % and pH was approx. 5.5. The thickness of Zn plating was determined as 5.93 μm by estimation of the passing charge amount applying 4 mA constant current.

Poly(aniline) (PANI), poly(pyrrole)(PPy), poly(o-anisidine)(POA) and poly(N-methylpyrrole)(PNMP) homopolymer films were synthesized electrochemically using cyclic voltammetry technique. After 48 h and 168 h of exposure time, electrochemical impedance measurements were obtained at measured open circuit potential values applying 7 mV of amplitude in frequency range from 105 to 10-3 Hz. The anodic polarization curves were recorded after 168 h of immersion time in corrosive test solution. The scan rate was 4 mV/s and the measured open circuit potential value was the initial potential for the scan. Scanning electron microscopy (SEM) was employed to characterize the surface structure with a JEOL JSM-5500LV scanning electron microscope.

## Results and Discussions

Zn plating on carbon steel (CS) was carried out applying 1, 2, 3, 4 and 5 mA constant current values in acidic chloride solution medium. Pt anode using for the plating was taken as 0.36 $cm^2$ surface area and coatings were obtained with stirring the solution open to atmosphere. It was observed that Zn plating obtained by applying 4 mA constant current value exhibited the better corrosion performance against the attack of corrosion products such as aggressive chloride ions to carbon steel electrode. In this study, Zn particles were successfully deposited on CS applying current of 4 mA with chronopotentiometry technique in which a constant current. As seen in Figure 1, while the bare CS electrode indicated the evidence of emery, average grain sizes of Zn particles, which has more homogeneous and smooth structure plated on carbon steel were observed using SEM images as similar measurement.

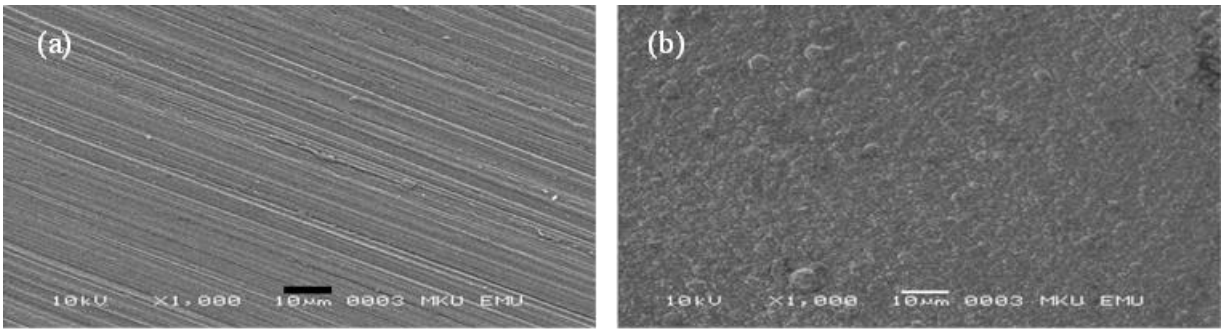


Figure 1. SEM images of CS (a) and CS/Zn (b) electrodes.

In order to improve the corrosion resistance of zinc (Zn) plating, four conducting homopolymer films were electrochemically synthesized on Zn plating surface by using cyclic voltammetric technique in a two-step process. So, polyaniline (PANI), polypyrrole(PPy), poly(N-methylpyrrole) (PNMP) and poly(o-anisidine) (POA) homopolymer films were electropolymerized on Zn deposited carbon steel (CS/Zn) electrode from sodium oxalate (NaOX) medium, which would enable the passivation of the CS/Zn surface using 10 mV/s scan rate in the potential range from -1.10 to + 0.40 V. In the second step, the film growth curves, which had seventy-five segments, were obtained using 150 mV/s scan rate in the potential range between 0.00 and 1.80 V (Figure 2). Although variation of potential range and scan rate was applied to obtain homogenous and smooth coating on zinc plated carbon steel, the highest anticorrosive coating was synthesized in condition given in this study.

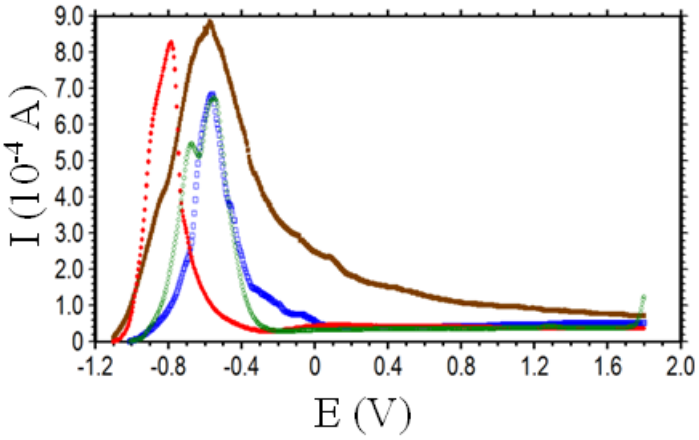


Figure 2. First CVs recorded for CS/Zn electrode in aniline (●), pyrrole (□), o-anisidine (o) and N-methylpyrrole (■) containing 0.20 M NaOX solution, scan rate: 10 $mVs^{-1}$.

In Fig. 3 given are the SEM images of CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes. SEM images clearly show that zinc plated carbon electrode was covered with a different homopolymer film of strongly adherent homogeneous crystalline structure.

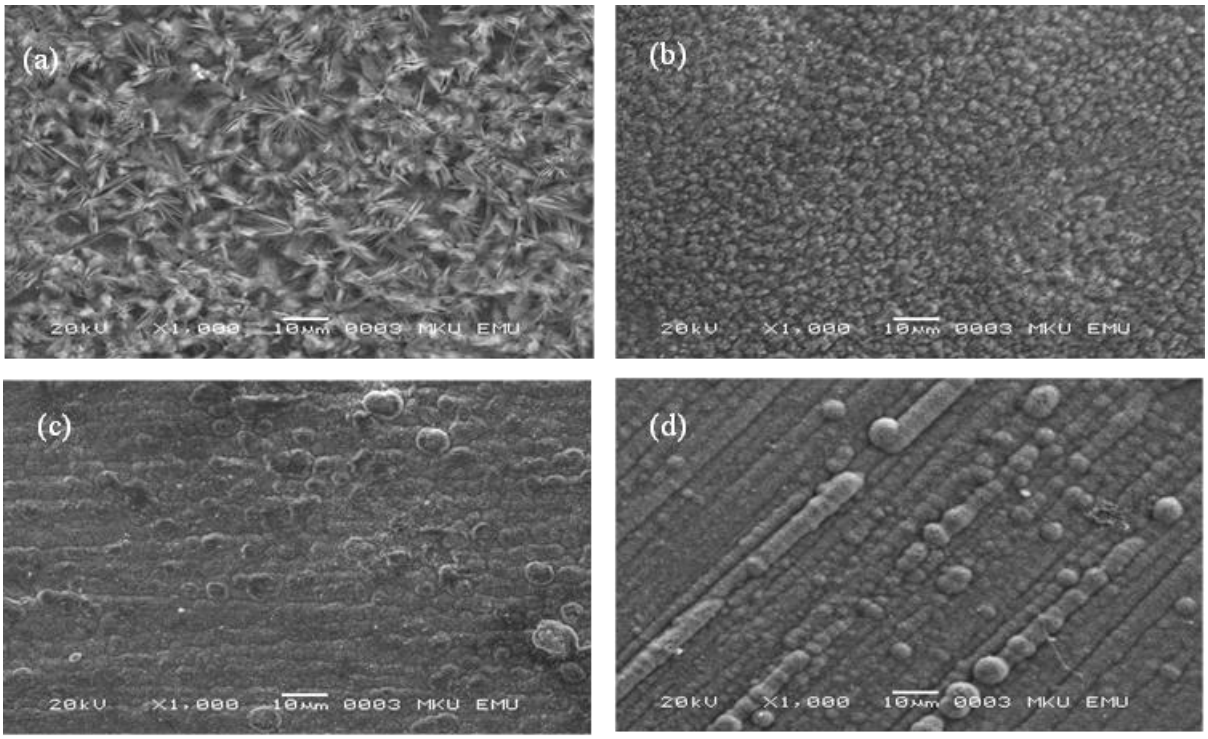


Figure 3. SEM images of CS/Zn/PANI (a), CS/Zn/PPy (b), CS/Zn/POA (c) and CS/Zn/PNMP (d) electrodes.

Linear sweep voltammograms for CS, CS/Zn, CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes in 0.05 M EDTA containing 0.50 M sodium sulphate solution are given in Figure 4. All measurements were taken at scan rate of 5 mV/s. While only single anodic peak for bare CS electrode was observed for the dissolution of iron and formation of iron complex with EDTA, there were two anodic dissolution peaks of zinc and iron at approx. -0.86 and 0.42 V, respectively, in presence of CS/Zn. The peak at the negative potential recorded was not seen for CS electrode. All of CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes exhibited two anodic dissolution peaks due to formation of zinc and iron complex with EDTA. It must be noted that the peak corresponding to the negative anodic dissolution in Fig. 4 was evidence for presence of zinc plating under polymer film coating. However, the dissolution peaks of homopolymer film coated CS/Zn electrodes had low intensity, corresponding to its significant barrier behavior against dissolution, while bare CS and CS/Zn electrodes exhibited high dissolution peak at the positive potential region. This event indicated that the top coated homopolymer films did not allow significant dissolution of CS and Zn plating on CS electrode surface. The lowest peak intensity was observed for CS/Zn/POA electrode, due to the effective barrier behavior of the POA film.

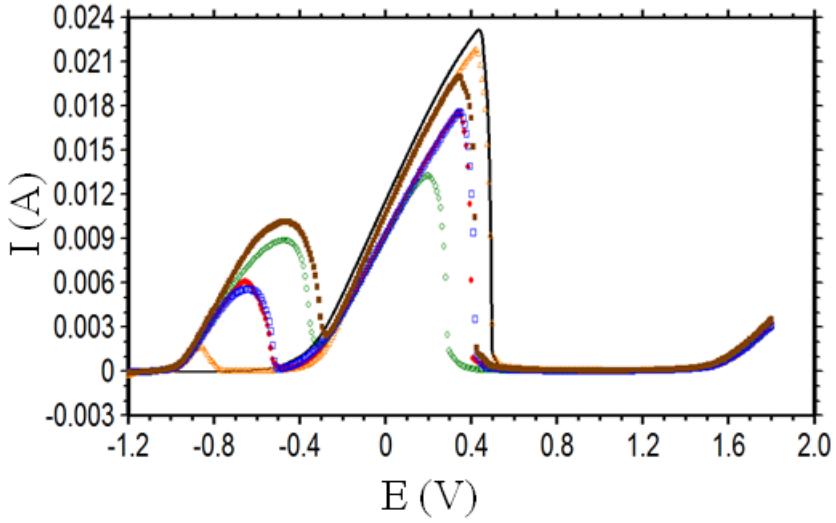


Figure 4. The linear sweep voltammograms recorded for CS (-), CS/Zn (Δ), CS/Zn/PANI (●), CS/Zn/PPy (□), CS/Zn/POA (o) and CS/Zn/PNMP (■) electrodes in 0.05 M EDTA containing 0.50 M sodium sulphate solution. Scan rate : 5 mV/s.

Immediately after the immersion time, the $E_{ocp}$-time curves obtained for CS, CS/Zn, CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes in 3.5 % NaCl solution are given in Figure 5. To determine the property of the Zn metal coated on the CS electrode, it was clearly seen that the Eocp value of the Zn-coated CS electrode was negative compared to the uncoated CS electrode. This behavior of Zn plated electrode showed the presence of a layer on CS electrode. In case of homopolymer film coated electrodes, the Eocp values of homopolymer film coated CS/Zn electrodes appeared to be at positive potential when compared with value of CS/Zn sample, immediately after the immersion time. The positive shift in the Eocp values obtained for PANI, PPy, POA and PNMP film coated CS/Zn electrodes simply indicated that Zn plating and these homopolymer films provided an adequate physical protection to metal between the corrosive environment and base metal. The Eocp values of CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes were cathodic direction due to their conductive structure and the presence of zinc in the polymer film pore base when compared with uncoated CS electrode.

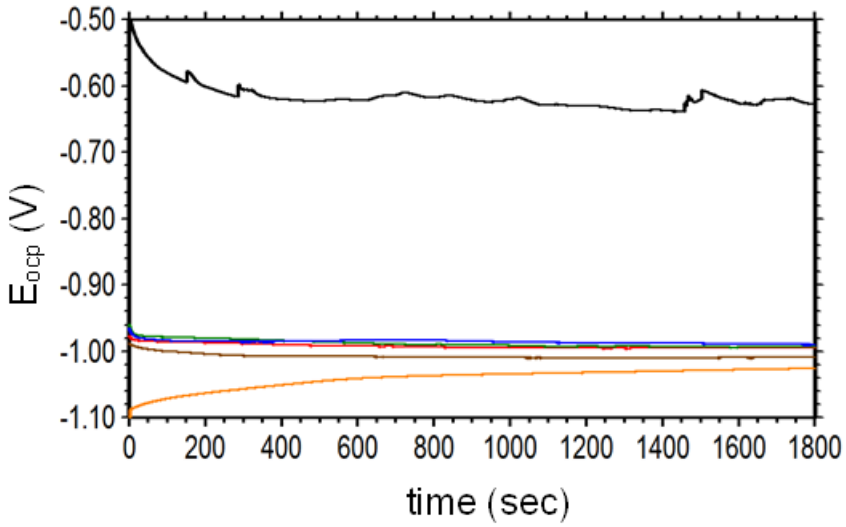


Figure 5. $E_{ocp}$-time curves recorded for CS (-), CS/Zn (Δ), CS/Zn/PANI (●), CS/Zn/PPy (□), CS/Zn/POA (o) and CS/Zn/PNMP (■) electrodes after exposure time in 3.5 % NaCl solution.

Anodic polarization curves obtained for CS, CS/Zn, CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes after 168 h exposure time in 3.5 % NaCl solution are given in Figure 6. In the case of uncoated CS sample, corrosion potential value ($E_{corr}$) was obtained to be -0.665 V. Current values increased so rapidly that there was not any possibility for passivation of the CS electrode surface under the aggressive chloride ions condition. The Ecorr value of CS/Zn electrode (-0.895 V) shifted in the active region due to zinc dissolution than that of uncoated CS electrode. It is clearly seen that CS/Zn electrode exhibited significantly highest current values. On the other hand, the Ecorr value of CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes were measured as -0.916, -0.893, -0.592 and -0.903 V, respectively. The positive shift in the Ecorr value for CS/Zn/POA electrode simply indicated that POA homopolymer film had less conductivity when compared with PANI, PPy and PNMP on CS electrode surface. The current values of CS/Zn/PANI electrode were significantly lower when compared with other homopolymer film coated Zn plated carbon electrode as well as Zn plated carbon steel and bare carbon steel electrode. This study revealed that PANI homopolymer film provided important corrosion protection to CS electrode.

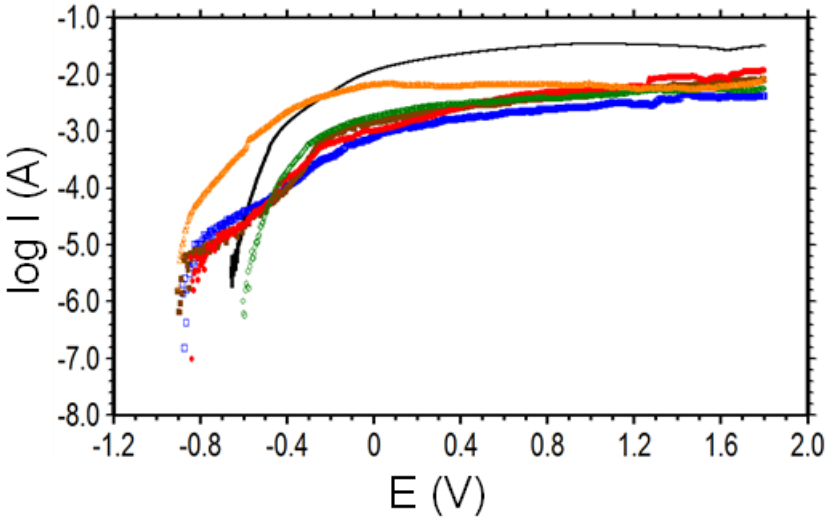


Figure 6. Anodic polarization curves recorded for CS (-), CS/Zn (Δ), CS/Zn/PANI (●), CS/Zn/PPy (□), CS/Zn/POA (o) and CS/Zn/PNMP (■) electrodes after 168 h of exposure time in 3.5 % NaCl solution, scan rate: 4 mV/s.

The Nyquist diagrams recorded for CS, CS/Zn, CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes obtained in 3.5 % NaCl solution are given in Figure 7, after 48 h and 168 h of exposure time. In Figure 7(a), Nyquist plots obtained for CS and CS/Zn electrodes consisted of one depressed semicircle ranging from high frequency to low frequency for both electrodes, after 48 h of immersion time. The diameters of these depressed semicircles were equal to polarization resistance ($R_p$). The depressed semicircle for bare CS electrode was equal to $R_p$ including the total of the charge transfer resistance ($R_{ct}$) that is responsible for the anodic dissolution of bare metal and diffusion resistance ($R_d$) (Walter, 1986; Mansfield 1995; Ozyilmaz, 2011). At this time, the $E_{ocp}$ value of bare CS electrode was found to be approx. -0.669 V, while that of CS/Zn electrode was -0.990 V. The Eocp value of CS/Zn electrode was cathodic direction when compared with CS electrode. The more negative $E_{ocp}$ value for the CS/Zn electrode indicated the presence of active zinc metal coating on the CS metal. On the other hand, indicated prominent differences between dissolution behavior of Zn plated layer and the corrosion reactions at the CS and CS/Zn surfaces. It has been observed that the Zn alloy coating exhibits a significant active layer appearance on the carbon steel against corrosive products. Consequently, this behavior observed for CS/Zn electrode within 48 h were an indication that the highest Rp value which was equal to the Rct and film resistance ($R_f$), was attributed to the total of Zn plating resistance ($R_{Zn}$) and oxide layer resistance ($R_o$) formed on the surface was observed for CS/Zn electrode (Walter, 1986; Mansfield 1995; Ozyilmaz, 2011). On the other hand, there was one depressed semicircle for PANI, PPy, POA and PNMP homopolymer film coated CS/Zn electrodes, after 48 h of exposure time. In the Nyquist diagram, the depressed semicircle which was equal to the $R_p$ value consisted of polymer film resistance ($R_{pf}$), RZn, Ro and Rct corresponding to dissolution of substrate at the bottom of the coating pores. This Rp value of CS/Zn/PANI electrode was higher when compared

with those of other coated CS electrodes and as well as CS/Zn. Moreover, the Rp value of CS/Zn/PNMP electrode was lowest due to high permeability and conducting property of homopolymer film, when compared with that of other homopolymer film coated CS/Zn electrodes, during this period. After 168 h of immersion time, decreasing in Rp values obtained for CS/Zn and bare CS electrodes were an indication that there was an increase in the amount of electrolyte solution within Zn plating and CS surface. Yet, the $R_p$ value recorded for CS/Zn electrode also was found to be lower than that of uncoated CS metal, after 168 h of exposure time. As seen from Figure 7(b), there was one depressed semicircle for CS/Zn/PANI, CS/Zn/PPy, CS/Zn/POA and CS/Zn/PNMP electrodes, after 168 h of exposure time. In the Nyquist diagram, the depressed semicircle which was equal to the Rp value consisted of $R_{pf}$, $R_{Zn}$, $R_o$ and $R_{ct}$ corresponding to dissolution of substrate at the bottom of the pores. This $R_p$ value of CS/Zn/PANI electrode was higher when compared with those of other coated CS electrodes. Moreover, the $R_p$ value of CS/Zn/PPy electrode was lowest due to high permeability and conducting property of homopolymer film, when compared with that of other homopolymer film coated CS/Zn electrodes, during this period.

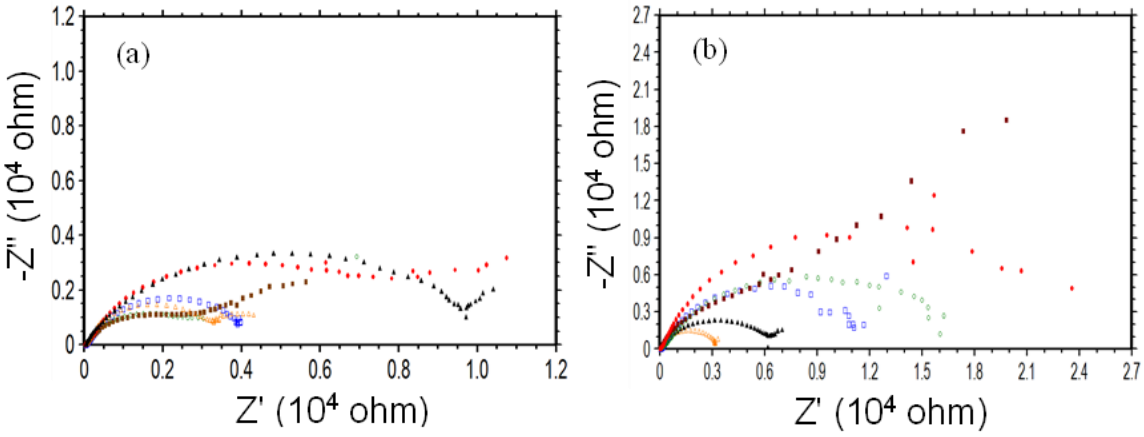


Figure 7. The Nyquist plots recorded for CS (▲), CS/Zn (Δ), CS/Zn/PANI (●), CS/Zn/PPy (□), CS/Zn/POA (o) and CS/Zn/PNMP (■) electrodes after 48 (a) and 168 h (b) of exposure time in 3.5 % NaCl solution.

In conclusions, according to results of SEM analysis, PANI, PPy, POA and PNMP coatings were successfully synthesized on CS/Zn electrode, applying cyclic voltammetry technique. Homogenous and adherent homopolymer films were produced using 0.10 M monomer solution containing 0.20 M sodium oxalate as electrolyte. It was found that the passivation of CS/Zn surface was necessary for homogenous homopolymer film synthesis prior to monomer oxidation and film growth. The corrosion performances of bare CS and CS/Zn electrodes were lower when compared with homopolymer film coated CS/Zn electrodes. PANI homopolymer film exhibited significant highest physical barrier behavior on Zn plated carbon steel, in longer exposure time.

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**Conflict of Interest**

The author declared that they have no competing interests.

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