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COPPER(II) BIOREMOVAL BY THERMOPHILE *CYANOBACTERIUM APONINUM*

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ABSTRACT. In the current study, bioremediation of Cu(II) by thermophile *Cyanobacterium aponinum* has been studied in BG11 media under different conditions. The optimum pH was 9 due to the maximum Cu(II) bioremoval efficiency as 71% in the medium with12.8 mg/L Cu(II). According to the results obtained from the trials, the highest bioremoval was 76.6% in the medium including 9.7 mg/L Cu(II) for incubation period of 10 days. When the effect of increasing temperature (25-45 °C) and biomass [20% and 40% (v/v)] concentrations on bioremediation by *C. aponinum* was investigated, the highest heavy metal removal was found 75.8% at 45 °C, 12.8 mg/L Cu(II), and 20% (v/v) biomass concentration. It was 76.3% in the medium with 13.8 mg/L pollutant, 40% (v/v) biomass concentration. The q_m (maximum specific Cu(II) removal) was found as 6.1 mg/g at 45 °C in BG11 with 40% (v/v) biomass and 13.8 mg/L Cu(II). It has been concluded that Cu(II) bioremediation by thermophile *C. aponinum* was firstly investigated at various environmental conditions in this study. The results indicated that the tested cyanobacterium had a great potential to remove heavy metals from the aquatic environments, containing Cu(II).

1. INTRODUCTION

Water is an indispensable resource for life on earth. Access to clean water is extremely important for all living forms. Recently, water quality has decreased in consequence of industrial activities, rapid population growth, unplanned urbanization, and overuse of natural resources. The spread of activities such as agriculture, industry, urbanization, and population growth cause many pollutants that to be toxic to the environment [1].

Heavy metal pollution is a critical problem owing to their harmful properties to people, animals, and plants [2]. On the other hand, these metals are non-degradable and having possibility of accumulation in organisms. The most common heavy metals presenting in industrial wastewaters might be copper, nickel, lead, uranium, chromium, arsenic, zinc, silver, cadmium, and iron [3]. Wastewaters containing heavy metals, which is given to the nature without treatment, join the ecosystem and could be accumulated in various organs of organisms and create toxicity. Among these heavy metals, presence of copper in excess causes serious problems. Therefore, copper-contaminated wastewaters must be remediated before discharging them to the environment [4].

Keywords. Cyanobacterium aponinum, heavy metals, thermophile, bioremediation, wastewaters
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There is a need for inexpensive, sustainable, and environmentally friendly methods for the bioremediation of metal-contaminated waters. Biological methods are preferred instead of chemical methods in heavy metal removal. Studies have shown that microorganisms with bioremediation capacity were very effective in removing metals from the environment [5]. Bioremediation is defined as the conversion of harmful and toxic substances by microorganisms into less toxic or non-toxic forms by microorganisms [6]. Prokaryotic and eukaryotic [7] microorganisms are used in the biological removal of heavy metals. Cyanobacteria are the largest photosynthetic prokaryotes and are capable of living in a variety of extreme habitats, from fresh and marine waters to terrestrial environments [8]. Cyanobacterial strains having a significant capability of heavy metal sorption were used in bioremediation processes [9]. Cyanobacteria have many features that make them preferred for the selective bioremoval and reduction of metals. These might be high removal capacity via absorption, tolerance to heavy metals by surviving in metal containing areas and having large surface area [10]. In recent years, extremophile microorganisms have attracted considerable interest. Among them, thermophile ones are preferred in many biotechnological applications.

Thermophile cyanobacteria have developed resistance pathways to adapt against several pollutants. These organisms have physiological and enzymatic properties to grow in undesirable conditions. These features make them as promising candidates for use in environmental biotechnological applications [11,12].

According to the previous studies, bioremediation of heavy metals done with cyanobacteria were mostly performed with mesophiles. De Philippis et al. [13] used *Cyanospira capsulata* and *Nostoc PCC7936* cyanobacteria producing exopolysaccharide for the bioremoval of heavy metals like nickel(II), copper(II), and zinc(II). In the same study, two cultures of cyanobacteria were shown to remove heavy metals, and it was stated that *C. capsulata* was more effective in bioremoval of heavy metals than other cyanobacteria, and was especially could tolerate Cu(II). In another study, the bioremoval of some metals [Zn(II), Cd(II), Cu(II), Pb(II)] by the secreted substances of *Cylindrospermopsis raciborskii* was investigated and it was found that the molecules secreted by the cyanobacteria showed the highest affinity for Cu(II) [14]. Heidari et al. [15] showed that when *Oscillatoria* sp. and *Leptolyngbya* sp. cyanobacteria were used as a consortium; consortia could remove chromium with the highest bioremoval capacity. In the same study, copper ions were removed with the lowest efficiency. In the study of Balaji et al., biological treatment of cadmium, chromium and lead heavy metals from leather industry wastewaters was carried out by Spirulina species [16]. In another study, Hazarika et al., showed bioremediation of heavy metals as Cu(II), Pb(II), Cd(II), and Zn(II) by *Noctoc muscorum* [17].

According to our knowledge is there is no study investigating Cu(II) bioremediation by thermophile *C. aponinum*. Therefore, the current study will be the first paper for the literature. The purpose of the study is to research Cu(II) bioremediation performance of thermophile *C. aponinum.* Effect of different environmental conditions such as pH, increasing metal concentrations, temperatures, and biomasses were studied on Cu(II) bioremediation capacity of *C. aponinum*.

2. MATERIALS AND METHODS

2.1. Cyanobacteria and culture conditions

C. aponinum was supplied from cyanobacteria collection of Biology Department, Faculty of Science, Ankara University [18]. Cyanobacterium was grown in containing 100 mL of BG11 media (pH 7) with using 250 mL Erlenmeyer [19] at 25 °C at a constant 2400 lx illumination in a climate cabinet [BINDER; KBW 400 (E5.1); 15–13640] for incubation period of 10 days.

2.2. Metal solution

Copper(II) stock solution was made by diluting $CuSO_4.5H_2O$ (Merck) to 1 g/L. The desired volumes of Cu(II) solutions were prepared from the stock Cu(II) solution.

2.3. pH Effect on Cu(II) bioremoval

pH media including nearly 10 mg/L Cu(II) was adjusted to 6, 7, 8, and 9 by using 1M NaOH and 1M HCL. Cu(II) concentrations were 10.8 mg/L (pH 6), 10.1 mg/L (pH 7), 9.8 mg/L (pH 8), and 12.8 mg/L (pH 9). Cyanobacterial cultures (20 mL) were added to 100 mL of media. After incubation for 2 days, the residual Cu(II) in the media was measured. Further trials were carried out at the optimal pH that was determined from these studies.

2.4. Effect of increasing Cu(II) concentration on Cu(II) bioremoval

In these trials, in the media at pH 9, the initial metal concentrations were 9.7, 14.2, and 22 mg/L. A 20% (v/v) biomass of cyanobacteria was inoculated into the prepared media. The remaining Cu(II) concentration was found after incubation for 10 day.

2.5. Effect of increasing temperatures and biomasses on Cu(II) bioremoval

For these experiments, different biomass concentrations [20% (v/v) and 40% (v/v)] of the tested cyanobacteria were used. Erlenmeyer flasks including approximately 15 mg/L Cu(II) at pH 9 were set at 25-45 °C.

In these experiments carried out with 20% (v/v) *C. aponinum* biomass, the concentration of Cu(II) was 15 mg/L at 25 °C, 14.6 mg/L at 35 °C, 12.8 mg/L at 45 °C. Copper(II) concentrations in media with 40% (v/v) biomass were 13.1 mg/L at 25 °C, 12.4 mg/L at 35 °C, and 13.8 mg/L at 45 °C. After incubation (10 days), the remaining Cu(II) concentrations were found.

2.6 Analytical methods

Three millilitres of samples were taken daily and centrifuged for 10 minutes at 6.000 rpm (Hettich®; EBA12; Germany) to remove the biomass. Microbial growth was followed by measuring the dry weight. The concentration of Cu(II) in the supernatant was found spectrophotometrically (Shimadzu UV 2001) at 460 nm by using sodium diethyl di-thiocarbamate as the complexing agent [20]. The BG11 medium without cell was used as the blank.

Copper(II) bioremoval yield was studied as a function of the pH, metal concentration, temperatures, and biomasses. The percentage of Cu(II) removal yield was found with Equation (1):

$$Y\% = \left(\frac{Co - Cf}{Co} \right) \times 100$$

Copper(II) bioremoval capacity can be measured based on the mass balance principle with Equation (2):

$$qm = [(Co — Cf)] / Xm$$

The maximal specific Cu(II) bioremoval (q_m) shows the maximal amount of heavy metal (mg) per unit dry weight of the cyanobacterial cells (g), the maximal dried cyanobacterial cell mass is *Xm* (g/L), and *Co* is the initial and *Cf* is the final concentration (mg/L) of Cu(II).

2.7. Statistical analysis

The results were subjected to analysis of the remarkable differences was performed by using variance method (ANOVA) and then compared by using standard deviations (±S.E.) The trials were done with two repetitions.

1. RESULTS AND DISCUSSION

3.1. Copper(II) bioremoval at different pH

The trials were performed in medium having with nearly 10 mg/L of Cu(II) at various pHs levels of 6, 7, 8, and 9 to determine appropriate pH level for the

maximum bioremoval of the pollutant (Table 1). Our results showed that, Cu(II) bioremoval by living *C. aponinum* biomass also increased depending on increase in pH. In the BG11 media including 12.8 mg/L Cu(II), the *C. aponinum* bioremoved heavy metal with the highest yield of 71% at pH 9. Due to the data obtained from these experiments, further trials were done in BG11 media at pH 9.As a result of statistical analysis, a significant difference was found in terms of bioremediation efficiency by using different pH.

Zinicovscaia et al. [21] studied the bioremoval of various metals like Zn, Cr, Cu, Fe, and Ni by *Spirulina platensis* in natural wastewaters. In that study, it was underlined that Cu(II) is in hydroxide form at pH 9.5, and therefore, metal bioremoval can only be done on the cell surface via microprecipitation. Sen et al. [22] showed that a consortium of *Limnococcus limneticus* and *Leptolyngbya subtilis* cyanobacteria removed 15 mg/L Cr(VI) with the highest efficiency at pH 9. In our study, *C. aponinum* had also the highest metal bioremoval at pH 9 as supported by the previous studies.

TABLE 1. Cu(II) bioremoval by *C. aponinum* at various pH (C_o: initial Cu(II) concentration, Y%: Cu(II) bioremediation yield; Biomass concentration: 20% (v/v); pH: 6–9; Temperature: 25 °C; Light intensity: 2400 lx; Incubation: 2 days; $p = 0.000$, F for different pH and yields: 98.3).

pH	Cu(II)	
	C_o (mg/L)	**Y%**
6	10.8	47.0 ± 1.0
7	10.1	57.0
8	9.8	66.0 ± 1.0
9	12.8	71.0

3.2. Copper(II) bioremoval at different pollutant concentrations

The trials were performed in BG11 media including nearly 10, 15, and 20 mg/L pollutants to investigate Cu(II) bioremoval by *C. aponinum*.

C. aponinum removed the applied 9.7 mg/L Cu(II) with a bioremoval yield as 61.6% after incubation for 3 days (Figure 1). In the same media, bioremediation of Cu(II) reached the maximum efficiency as 76.6% at the end of 10 days. In media including 14.2 mg/L Cu(II), the tested cyanobacterium removed 61.5% of the pollutant after incubation for 3 days; bioremoval yield was 71.5% after incubation for 10 days.

When the Cu(II) concentration was 22 mg/L, *C. aponinum* was successfully removed the applied pollutant with a yield as 71.0% at the end of the incubation period.

Panta et al. [23] used *A. variabilis* GITAM RGP to remove Cu(II) and other heavy metals from different wastewaters; they found 87.5% Cu(II) bioremoval efficiency at a very low metal concentration as 1.93 mg/L Cu(II). Fawzy [24] investigated the bioremoval of Cu(II) and cadmium(II) by *Merismopedia tenuissima;* the bioremoval of Cu(II) was 52% in the medium containing 1.2 mg/L Cu(II). In the same study, it was determined that the bioremoval efficiency decreases with the rise of metal concentration. In the present study, there was a slight decline in bioremoval efficiency with a rise in heavy metal concentration.

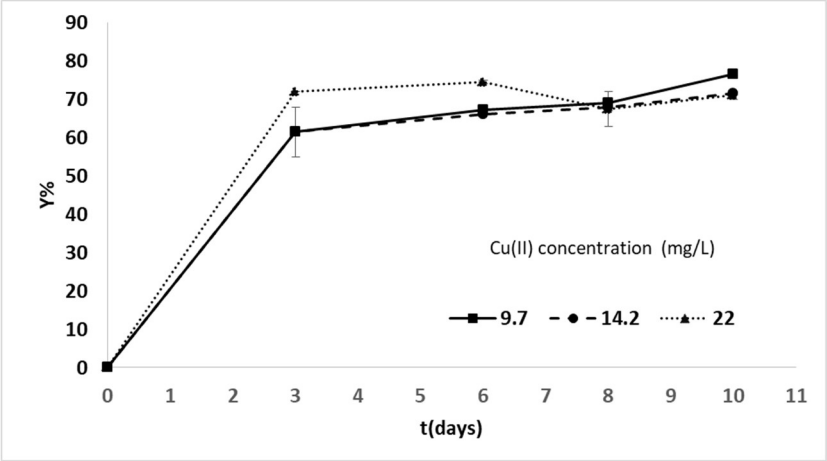

FIGURE 1. Cu(II) bioremoval by *C. aponinum* at increasing Cu(II) concentrations (Biomass concentration: 20% (v/v); Light intensity: 2400lx; pH 9; Temperature: 25 °C; Incubation: 10 days; $p < 0.05$, F for increasing Cu(II) concentrations and days: 0.229).

Cyanobacterial growth was not inhibited in the medium with increasing Cu(II) concentrations and was found at similar rates. However, as the metal concentration increases, the number of functional groups to which the metal is attached is similar. According to a previous study, there is a certain saturation capacity in removing metals from the environment and metal bioremoval capacity becomes saturated after a certain concentration of the pollutant [25]. In our study, as the metal concentration is increased, the number of functional groups to which the metal will be attached was similar in all tested media. Therefore, Cu(II) bioremoval slightly decreased in the media with increasing heavy metal. In addition, according to statistical data, when the experiments with increasing copper concentrations were evaluated, there was no significant difference between the concentrations in terms of removal efficiency.

In the current study, we found higher efficiency of pollutant removal at higher initial Cu(II) concentrations than the mentioned studies, and heavy metal bioremediation was achieved above 71% in the media with all the Cu(II) concentrations tested.

3.3. Copper(II) bioremoval at different temperatures and biomass concentrations

In the media including 15 mg/L Cu(II) at 25 °C, the highest Cu(II) bioremoval was 70.5% in 20% (v/v) biomass-samples after 10 days incubation (Figure 2). With the increment in temperature, Cu(II) bioremoval rose to 72.6% in 20% (v/v) biomass and 14.6 mg/L Cu(II) after 10 days incubation. At 45 °C, there was a slight increment of the bioremediation yield, and the percentage of efficiency was 75.8% after incubation for 10 days.

The increment of biomass from 20% (v/v) to 40% (v/v), Cu(II) bioremoval yield increased from 70.5% to 71.5% in medium with 13.1 mg/L heavy metal at 25 °C after incubation for 10 days (Figure 3). In the media at 35 °C, the maximum Cu(II) bioremoval was 72.9 % in the BG11 media having 40% (v/v) biomass and 12.4 mg/L Cu(II) after incubation for 10 days. The bioremoval yield was found as 76.3% in the medium at 45 °C with 40% (v/v) biomass and 13.8 mg/L heavy metal concentration after incubation for 10 days.

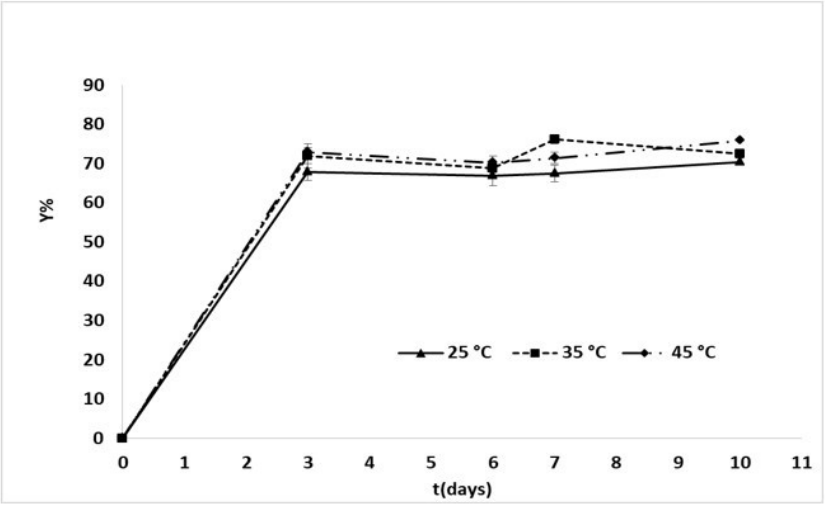

FIGURE 2. Cu(II) bioremoval by *C. aponinum* at increasing temperatures (C_o-25 °C:15 mg/L, C_o-35 °C: 14.6 mg/L, C_o-45 °C: 12.8 mg/L; Biomass concentration: 20% (v/v; Light intensity: 2400lx; pH 9; Temperatures: 25-45 °C; Incubation: 10 days; $p < 0.05$, F for increasing temperatures and days: 4.18).

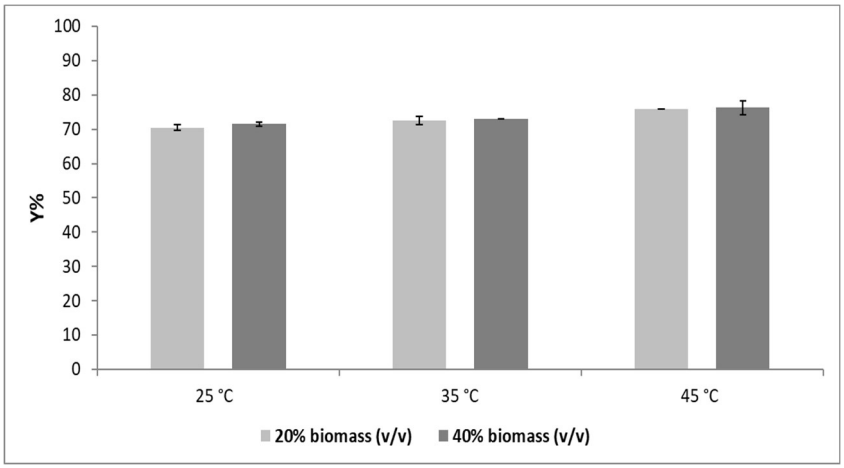

FIGURE 3. Comparison of Cu(II) bioremoval in BG11 with 20% (v/v) and 40% (v/v) biomass (C_o: nearly 15 mg/L; Light intensity: 2400lx; pH 9; Temperatures: 25-45 °C; Incubation: 10 days; $p < 0.05$, F for increasing biomass and yields: 0.102).

Like in the current study, Zinicovscaia et al. [26] emphasized that the bioremoval efficiency did not increase at the same rate by increasing the amount of cyanobacterial biomass from 10 g/L to 60 g/L. In that study, with using 6 times higher biomass, efficiency percentage only increased from 61% to 83%. Due to the statistical results, there was no meaningful difference for bioremoval efficiencies between 20% and 40% biomass at the mean 0.05 significance level.

In the medium containing approximately 15 mg/L Cu(II), the heavy metal bioremoval of cyanobacteria increased with the increase of the temperature from 25 to 45°C. Cyanobacterial growth was not affected negatively from the temperature increment and the growth rate of *C. aponinum* was similar at all the tested temperatures in the media with pollutant. According to the statistical data, a significant difference was found when bioremediation efficiencies at 25 °C compared with the trials performed at other temperatures.

The optimum growth temperature for *C. aponinum* has been previously shown to be between 35-40 °C in contaminant-free environments [27, 28]. In the current study, when *C. aponinum* was exposed to two stress conditions such as heavy metal and temperature, the cyanobacterium continued its growth in that media and performed effective heavy metal bioremoval. Cyanobacteria are known to synthesize small cysteine-ric proteins called metallothionein, which can bind toxic heavy metals [29]. In our study, *C. aponinum*, which was exposed to temperature stress in heavy metal-containing media, may have increased heavy metal bioremoval by synthesizing these types of proteins.

TABLE 2. The q_m values in the media with different biomass concentrations and temperatures (Biomass concentration: 20% (v/v), 40% (v/v); pH: 9; Light intensity: 2400 lx; Incubation: 10 days; in media with 20% (v/v) biomass, $p < 0.05$, F for at different temperatures and q_m: 14.9; in media with 40% (v/v) biomass, $p < 0.05$, F for at different temperatures and q_m: 14.9).

20% biomass (v/v)		
T (°C)	**C_o (mg/L)**	**q_m (mg/g)**
25	15.0	12.3 ± 0.4
35	14.6	13.5 ± 0.3
45	12.8	11.9 ± 0.01
40% biomass (v/v)		
T (°C)	**C_o (mg/L)**	**q_m (mg/g)**
25	13.1	5.3 ± 0.2
35	12.4	5.6 ± 0.6
45	13.8	6.1 ± 0.2

In the medium with 15 mg/L Cu(II), %20 (v/v) biomass at 25 °C, the q_m was 12.3 mg/g. In %20 (v/v) biomass-samples at 35 °C and 45 °C, the q_m values were 13.5 mg/g and 11.9 mg/g in the medium with 14.6 mg/L and 12.8 mg/L Cu(II), respectively. In BG11 medium with 40% (v/v) biomass and 13.1 mg/L Cu(II), q_m was 5.3 mg/g at 25 °C. Copper bioremoval per one gram of the *C. aponinum* biomass was 5.6 mg/g in the medium with12.4 mg/L Cu(II) and 40% (v/v) biomass at 35 °C. The q_m was 6.1 mg/g in samples with 13.8 mg/L Cu(II) and 40% (v/v) biomass at 45 °C.

When the q_m values were evaluated statistically in the experiments carried out at increasing temperatures and biomasses, a significant difference was found.

Copper(II) bioremovals per one gram of the cyanobacterial biomass increased in samples with increasing temperatures. In the BG11 with Cu(II), bioremoval yields increased with an increase in temperature, therefore, the maximum specific heavy metal bioremovals increased. The maximum specific Cu(II) bioremovals were found to be lower in samples with 40% biomass (v/v) compared to samples with 20% biomass (v/v). Since the biomass is doubled, a decrease in Cu(II) removal per 1 gram of biomass is an expected result. There was one exception in the 20% (v/v) biomass-samples at 45 °C that q_m decreased to 11.9 mg/g. This is because there was slightly more biomass at 45 °C than any other temperature tested. Thus, Cu(II) bioremoval per 1 gram of the biomass decreased. The maximum specific Cu(II) bioremovals were found to be lower in samples with 40% (v/v) biomass compared to samples with 20% (v/v) biomass.

Similar bioremoval percentages were found in the media with different biomass concentrations, therefore, q_m decreased.

At the end of these experiments, it was showed that an increment in the biomass concentration of *C. aponinum* and temperature had a favorable effect on the bioremediation of Cu(II). Statistical analysis showed that Cu(II) bioremediation by *C. aponinum* was mostly affected by pH and temperature.

4. CONCLUSIONS

In the current study, thermophile *C. aponinum* was used to bioremove Cu(II) on harsh conditions like alkaline media and high temperatures. The highest Cu(II) bioremoval by *C. aponinum* was found at pH 9. *C. aponinum* could grow at all the applied temperatures (25-45 °C) and it also had bioremoval capacity in samples with Cu(II). In BG11 medium with 13.8 mg/L Cu(II) and 45 °C, *C. aponinum* biomass [40% (v/v)] had the maximum bioremoval yield as 76.3%. The maximum specific Cu(II) bioremoval (q_m) was 13.5 mg/g in samples containing 14.6 mg/L Cu(II) and 20% (v/v) at 35 °C.

The results demonstrated that the thermophile *C. aponinum* tested in the present study had bioremediation capacity for the removal of Cu(II) from water. It was concluded that thermophile *C. aponinum* is a remarkable biologic sorbent with efficient heavy metal bioremediation capacity that might be applied in biological treatment of wastewaters.

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Author Contribution Statements SŞ- data collection, management, and manuscript writing. NKK- project development, data analysis, manuscript editing, manuscript writing. GD- project development, data analysis. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

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