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Dear Readers and Authors,

As “International Journal of Science Letters (IJSL)”, we are pleased and honored to present the first issue of 2023. IJSL, is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

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With these thoughts, we are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in this issue of International Journal of Science Letters.

26.03.2023

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Research Article

Prevalence of tuberculosis and rifampicin resistant M*ycobacterium tuberculosis* among patients receiving treatment in ercc medical center alushi, Nasarawa state, Nigeria

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Abstract

This study carried out a hospital based retrospective review of patients who had been screened for *Mycobacterium tuberculosis* (MTB) and Rifampicin resistant- Tuberculosis between January 2017 and July 2021 at the ERCC Medical Center, Alushi, a tuberculosis referral facility in Nasarawa state. The study included 3029 patients suspected of having TB, and the overall TB prevalence was 43.57% (1530 cases). Of the patients, 64.90% (933) were male, and 2.31% had rifampicin resistance. Meanwhile, 35.09% (537) were female, and 7.07% had rifampicin resistance. The highest TB prevalence was observed in the age groups 21-30 and 31-40, with 26.51% (350 cases) and 24.77% (327 cases), respectively. The study revealed that females and patients aged 21 to 40 were at a higher risk of developing rifampicin-resistant TB. Early identification of drug-resistant TB and prompt treatment is crucial to prevent its spread in the community. Community health education programs should be continuously implemented to raise awareness about TB and the risks of drug resistance, improving prevention and control efforts.

Article History

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Keywords

Mycobacterium tuberculosis,
Prevalence,
Rifampicin Resistant Tuberculosis,
Nasarawa State.

1. Introduction

The *Mycobacterium tuberculosis* Complex (MTC) is a bacterial family that causes the chronic infectious disease tuberculosis (TB) (Ukwamedua *et al*., 2019). Tuberculosis remains a severe public health concern since one-third of the world's population has latent TB and 10% of this group gets active disease during their lifetime (Nhamoyebonde *et al*., 2014). The spread of drug-resistant strains has aggravated tuberculosis prevention, diagnosis, and treatment, making it one of the main causes of morbidity and mortality among infectious diseases worldwide (WHO, 2018). Tuberculosis risk factors include childhood, old age, malnutrition,

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cigarette smoking, and alcohol intake (Olatunji *et al*., 2022). Furthermore, those with immune-suppressive disorders (such as HIV/AIDS) or who take immune-suppressing medications (such as cancer patients, organ transplant recipients, and people with autoimmune diseases) are more prone to contract tuberculosis (WHO, 2019; Olatunji *et al*., 2022).

Nigeria is one of 14 countries with a high prevalence of tuberculosis (TB), multi-drug resistant tuberculosis (MDR-TB), and tuberculosis-HIV co-infection (TB/HIV) (WHO, 2016). Nigeria is also one of five countries that contributed to a more than 60% discrepancy in the projected number of incident MDR/RR-TB patients and MDR-TB enrollment (WHO, 2016; Adejumo *et al*., 2018). A meta-analysis of the prevalence of drug-resistant tuberculosis (DR-TB) in Nigeria found MDR-TB in 6% of newly diagnosed patients and 32% of those who had previously been treated (Adejumo *et al*., 2018). Rifampicin is one of the most commonly used medications to treat tuberculosis (Reddy and Alvarez-Uria, 2017). It is less toxic and one of the two primary medicines used in short-term chemotherapy (0–6 months). As a result, patient compliance increases and, the need for protracted treatment, which normally lasts 18-24 months (Ikuabe and Ebuenyi, 2018), is eliminated. Mono-resistance to rifampin is uncommon. It is commonly seen in the presence of isoniazid resistance. As a result, MDR-TB accounts for over 90% of rifampicin-resistant TB (Jaleta *et al*., 2017; Ukwamedua *et al*., 2019).

The development or acquisition of DR-TB has been strongly correlated with prior TB treatment (Ukwamedua *et al*., 2019). However, other factors such as the use of inferior drugs, past exposure to quinolones, poor adherence to anti-TB medications, restricted access to healthcare, long-term chemotherapy linked to rifampicin-resistant TB, and a high prevalence of HIV/AIDS are all thought to have a role (Oneydum *et al*., 2017; Ukwamedua *et al*., 2019). Limited literature and data are available for rifampicin resistant TB for the study region. This study aims to measure the prevalence of Tuberculosis and Rifampicin resistant *Mycobacterium tuberculosis* among patients receiving treatment at ERCC medical Center Alushi, Nasarawa State from 2017 to 2021.

2. Materials and Methods

2.1. Study Design and Area

Hospital based retrospective review of presumptive TB register of patients screened for *Mycobacterium tuberculosis* (MTB) and RR-TB using Xpert MTB/RIF assay between January, 2017 and July, 2021 in ERCC Medical Center, Alushi. A Tuberculosis referral center in Nasarawa state, Nigeria.

2.2. Data Collection

Data collected includes age and sex of the patients who attended ERCC Medical Center, Alushi from January 2017 to July 2021. Included were all cases who had all these variables. A case was excluded if one of the variables was absent. Therefore, out of 3,577 patients' records examined, only 3029 were considered.

2.3. Data Analysis

The information was collected into data sheets in Microsoft Excel Version 2013. Results were displayed in form of descriptive statistics, tables and charts.

3. Results

In the present study, the prevalence of *M. tuberculosis* infection was found to be 43.57%. Table 1, shows the prevalence of Tuberculosis and Rifampicin resistant *Mycobacterium tuberculosis* stratified by sex. The data obtained show that males had higher TB infection 993(64.90%) than females 537(34.09%) but Rifampicin resistant *Mycobacterium tuberculosis* were higher in females 38(7.07%) than males 23(2.31%).

Table 1. Prevalence of Tuberculosis and Rifampicin resistant *Mycobacterium tuberculosis* stratified by sex

	2017		2018		2019		2020		2021		Total	
	+	RR	+	RR	+	RR	+	RR	+	RR		RR
Male	264	14(5.3)	207	1(0.5)	195	3(1.5)	194	4(2.0)	132	1(0.8)	993(64.90)	23(2.31)
Female	117	8 (6.8)	133	14(10.5)	123	8(6.5)	98	2(2.0)	66	6(9.1)	537(35.09)	38(7.07)
Total	381	22(5.8)	340	15(4.4)	318	11(3.4)	292	6(2.1)	198	7(3.5)	1530	7(3.5)

*values in bracket in percentage (%)
+: Positive RR: Rifampicin resistant

Figure 1 shows the prevalence rate of Tuberculosis from 2017 to 2021. As seen in the figure, it is shown that there is a downward trend in the prevalence of tuberculosis and Rifampicin resistant *Mycobacterium tuberculosis* from 2017 to 2021.

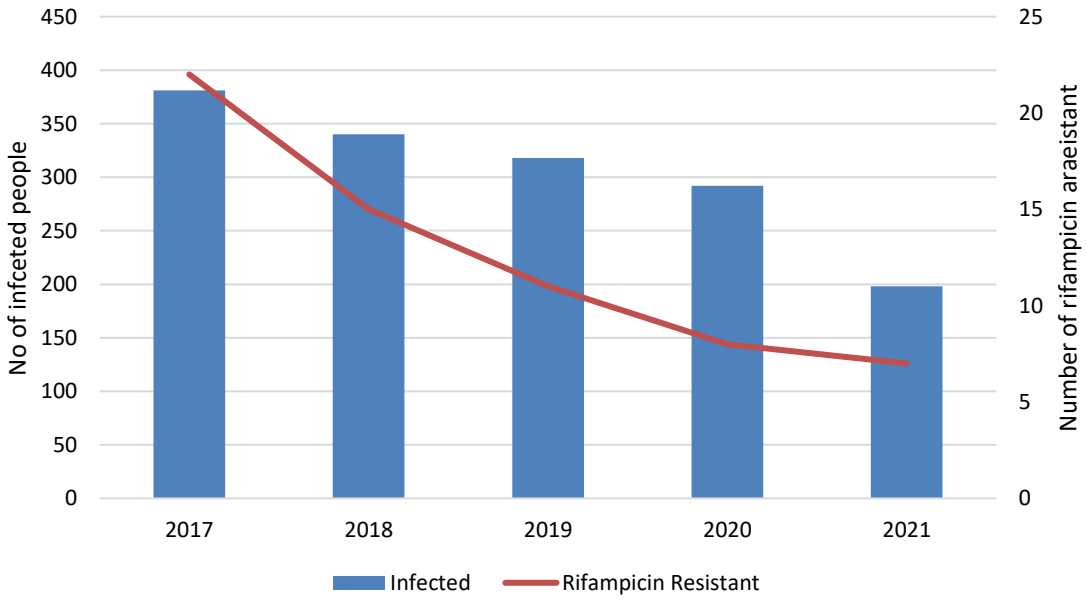

Figure 1: Chart showing the prevalence rate of Tuberculosis and Rifampicin Resistance from 2017 to 2021

Figure 2 shows the prevalence of Tuberculosis stratified by age from 2017 to 2021. The data presented shows that TB infection was higher within the age range 21-40 and least within the age range 70 and above.

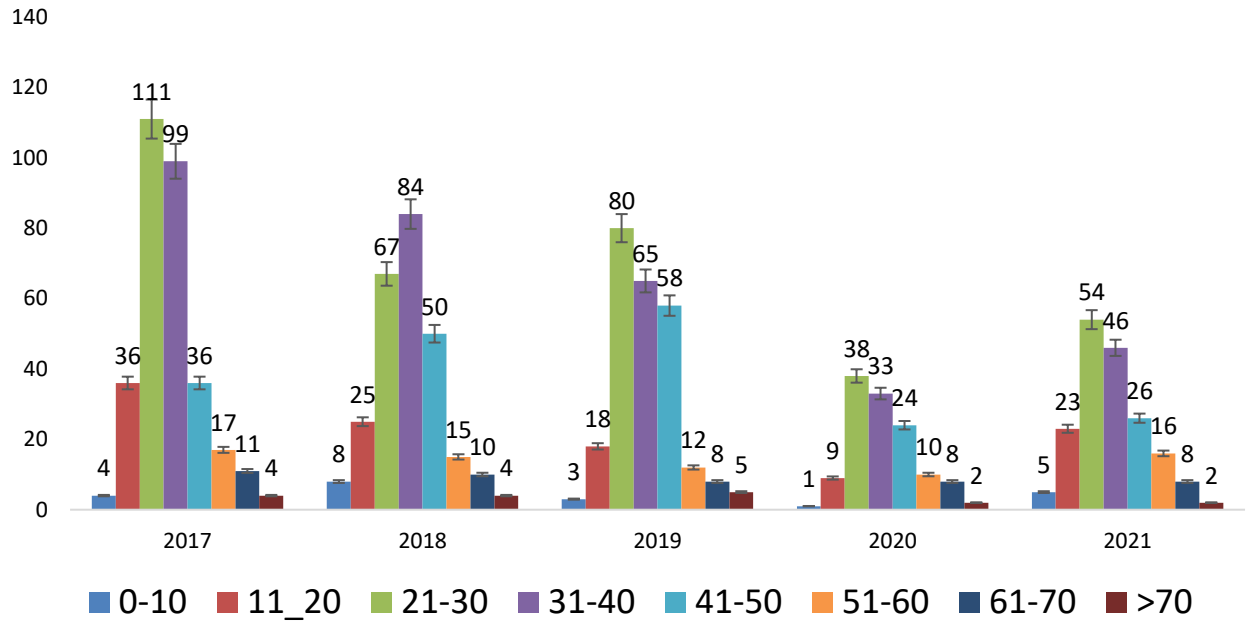

Figure 2: Chart showing the prevalence rate of Tuberculosis stratified by age

4. Discussion

In the current study, the prevalence of *M. tuberculosis* infection was found to be 43.57%. This finding outperforms studies from Ethiopia (Adane *et al*., 2015), Burundi (Sanders *et al*., 2006), and Nigeria's southern (Kuyinu *et al*., 2018) and northern regions (Audu *et al*., 2017). However, studies from a different Southern Nigerian state revealed a higher prevalence than that seen in this study (Osman *et al*., 2016). A systematic evaluation of the prevalence of DR-TB in Nigeria found that the disease varied across geographical regions, settings, and treatment approaches (Onyedum *et al*., 2017). The higher incidence found in this study could be related to the study location. ERCC Medical Center, Alushi, is a tuberculosis referral hospital in Nasarawa State. Patients with suspected DR-TB are usually referred from other hospitals in the state and beyond to Xpert MTB/RIF. In addition, as shown in figure 1, there is a downward trend in the prevalence of tuberculosis and Rifampicin resistant *Mycobacterium tuberculosis* from 2017 to 2021. The reducing trend in the prevalence of DR-TB in this study may be due to the increase in the number of Xpert MTB/RIF sites in the state (Audu *et al*., 2017).

In contrast to previous Nigerian research (Daniel and Osman, 2011; Kuyinu *et al*., 2018) that claimed there was no gender difference in tuberculosis and Rifampicin resistant tuberculosis prevalence, there was a gender difference in tuberculosis and Rifampicin resistant tuberculosis prevalence in this study. From 2017 to 2021, the study found a rise in tuberculosis infection among men compared to women, but a rise in RRTB among women compared to men (Table 1). However, research from the Northern and Southern regions of Nigeria revealed that males had a much greater prevalence of DR-TB (Tilako *et al*., 2013; Uzoewulu *et al*., 2014; Audu *et al*., 2017). The increased prevalence of tuberculosis among men was attributed to poor health seeking behavior in men, social stigma, and cultural practices (Uzoewulu *et al*., 2014). According to other research conducted in the country, lack of control over finances, low tuberculosis knowledge, delay in seeking medical attention, and poor health seeking behavior in females are all socioeconomic factors that may contribute to the prevalence of RR-TB in females (Wondimu *et al*., 2007; Storla *et al*., 2008).

Numerous studies have shown that prior TB treatment history is a significant risk factor for MDR-TB (Daniel and Osman, 2011; Chuchottaworn *et al*. 2015; Gunther *et al*., 2015; Fregona *et al*., 2017). This could be due to the use of substandard anti-TB drugs, frequent and inappropriate medication usage and, poor management of drug-sensitive TB.

Patients aged 21 to 40 years old had a higher risk of developing tuberculosis and Rifampicin-resistant TB than patients of any other age group in this study. This could be because this is the age range in which people are most likely to engage in social activities including sex on a regular basis. This age group is more social and outgoing, and they regularly engage in small conversations. This is similar to the findings from a study conducted in North East Nigeria (Tilako *et al*., 2013). According to surveillance data from Central and Eastern Europe, young adulthood is the peak age for MDR-TB prevalence (Workicho *et al*., 2017), which is in line with the findings of this study (Table 2).

Due to the retrospective nature of this study, the author could not control for potential confounding factors that may influence the development of rifampicin-resistant tuberculosis, such as comorbidities, socioeconomic status, and previous treatment.

5. Conclusion

According to this data analysis, tuberculosis and Rifampicin-resistant tuberculosis are common in persons of all sexes and ages. Rifampicin resistance was found to be significantly

associated with female gender. The fact that women are the majority of children's primary caregivers in our country may render children more vulnerable to RR-TB. This study's findings are incredibly significant and instructive, and they may aid health professionals and policymakers in understanding the problem and developing solutions regardless of its limitations. The study highlights the urgent need for early identification and prompt initiation of DR-TB medication to prevent the spread of the disease in the community. Community health education programs on tuberculosis and the risks of drug resistance should also be continuously implemented to improve prevention and control efforts. However, the study's limitations must be taken into consideration when interpreting its findings and developing future interventions. Further research is necessary to validate these findings and assess the effectiveness of current treatment strategies.

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Research Article

A toxicity study on *Daphnia magna* and *Artemia salina*: Are paper cups safe?

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Abstract

The objective of our study is to reveal the effects of paper cups sold under different brands on the aquatic test organisms, *Daphnia magna* and *Artemia salina*, which are frequently used in toxicity studies. To this end, survival rates of *D. magna* and *A. salina* individuals, which were kept alive after cooling in paper cups exposed to 20 °C and 80 °C, for 24, 48, 72, and 96 hours were determined.

Upon examining the results, while no significant decrease was found in the survival rates of *D. magna* and *A. salina* individuals kept in glassware, a significant increase was identified in the mortality rates of individuals kept in paper glasses, especially at 72 and 96 hours. It was determined that whereas the mortality rates reached 40% in paper and plastic cups exposed to 20 °C water, the mortality rates reached 70% in paper cups exposed to 80 °C water. Moreover, regression was found in the developmental and reproductive characteristics of *D. magna* and *A. salina* individuals, which were kept in paper cups exposed to water at different temperatures, compared to the control group and individuals in glass cups. We think that this was caused by microplastics or some chemicals released into the water due to the deterioration of the film layer on the inner surface of the glass, especially due to the high temperature.

Article History

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Keywords

Paper cup, toxicity, microplastics, *Daphnia magna*, *Artemia salina*

1. Introduction

Plastics are materials that have become indispensable elements of our daily life and are utilized in numerous fields (Akçay et al., 2020). Nowadays, only 10% of plastics used in large quantities can be recycled (Aydın et al., 2019). Plastics of different sizes can accumulate in the environment as a result of the degradation of plastics. Nanoplastics are formed as a result of the degradation of plastics in nature over time.

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The fact that microplastics are mistaken for food by aquatic organisms in water sources and swallowed and they can absorb permanent organic pollutants can be listed among the most important effects of microplastics. It has been reported that more than 140,000 living things, such as birds, sea turtles, whales, and dolphins, have died every year since the 1990s due to plastic exposure (Yurtsever, 2015). Microplastics, which can be classified in many different ways, can be classified as primary and secondary microplastics according to their sources. While primary microplastics are grouped as microbeads and textile fibers originating from cosmetics and personal care products, secondary microplastics can be defined as plastics that have passed through certain degradation stages of plastic waste in the environment (Esmeray and Yurtçu, 2020).

The sizes of microplastics, which are commonly found in the environment, vary due to different transformation processes, such as biodegradation and decomposition, which change their physicochemical properties (Ahmed et al., 2022). Microplastics are commonly classified as film (thin-layer plastic), foam (foam plastic), fragment (small particle plastic), fiber (fibrous plastic), pellet (round plastic), and granule according to their shapes (Virsek et al., 2016). Paper and cardboard are among the most common or preferred types of food packaging materials. They have gained popularity in the food packaging process due to their easy availability, affordability, lightweight, and success in acting as barriers to moisture, oxygen, and microbial entities. Disposable paper cups are among such food containers commonly used. It is a popular option for many people when consuming their favorite beverages. Disposable paper cups are used at most coffee and tea points of sale worldwide (Poortinga & Whitaker, 2018). Disposable paper cups are made from 90 - 95% (by weight) paper, and the remaining 5-10% (by weight) are a hydrophobic plastic film (Arumugam et al., 2018). The inner layer is usually made of polyethylene (PE), and sometimes copolymer alternatives are used (Rogovina et al., 2013).

Many previous studies have demonstrated that harmful chemicals and substances can leach from paper and cardboard-based food packaging into food intended for human consumption (Vandermarken et al., 2019). Various additives are used to process plastics, and layering provides the paper with the desired properties such as flexibility, color, and protection from microbial activity. Some organic compounds used in the processing of paper and cardboard food packaging have also shown their potential to migrate into packaged food (Xue et al., 2019). Phthalate compounds, such as di(2-ethylhexyl)phthalate and di-n-butyl phthalate, are plasticizers used during the production of paper and cardboard packaging, and these compounds increase the flexibility and durability of packaging materials (Hahladakis et al.,

2018). Antioxidant additives, such as phenolics and organophosphates, are utilized to delay the degradation process in polymers (Hahladakis et al., 2018). Fluoride-based compounds, such as perfluoroalkyl substances (PFAs), are used to make cardboard and paper waterproof and oil-repellent (Schultes et al., 2019). Heavy metal leakage has been identified from paper-based food packaging into food (Elmas et al., 2018). Even biopolymers utilized in food packaging are combined with nano-fillers, such as silicates and carbon nanotubules, to reduce their crystallinity and increase flexibility (Souza & Fernando, 2016). However, concerns about the leaching of microplastics from these food packaging materials have rarely been addressed or quantified. Microplastics have been identified in many foodstuffs such as salt (50-280 Microparticles/kg salt) (Sathish et al., 2020), branded milk (6.5 ± 2.3 particles/L) (Kutralam-Muniasamy et al., 2020), fish and other seafood (Sathish et al., 2020), and tea bags (11.6 x 109 microplastic/plastic tea bag) (Hernandez et al., 2019). Because disposable paper cups are among the most common forms of food packaging, the potential for plastic particles to migrate into hot beverages requires significant attention. Fresh and saltwater organisms were used in the study to assess the possible effects of paper cups of different brands sold in the market. Many different models of organisms, such as fish, microorganisms, plants, and invertebrates, have been utilized in numerous scientific studies investigating the effects of aquatic ecosystem pollutants (Kado et al., 1998). Zooplankton is mostly used in ecotoxicological experiments because it is at the origin of the food chain and is sensitive to chemicals (Hanazato, 1998). Water fleas, an important link in the food chain in aquatic environments, are aquatic test organisms frequently utilized in toxicology studies. Daphnia species, used as indicator organisms in toxicology studies, are very important due to their rapid growth rates, high reproductive potential, and short life cycles (Altındağ et al., 2008). *D. magna*, which can be found in stagnant regions of streams and lakes almost all over the world, is usually less than 3 mm in size (Tatarazako and Oda, 2007) (Figure 1.1).

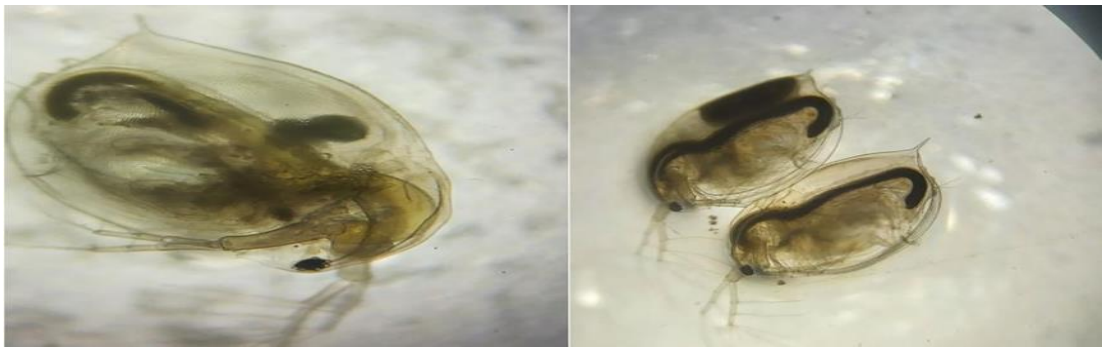

Figure 1.1. *Daphnia magna* (Original)

In addition to its key role in the aquatic food chain, it primarily feeds on bacteria and algae under natural conditions (Li and Tan, 2011) and is the most important food source for predatory fish and invertebrates (macro arthropods) (Li and Tan, 2011). Because of its importance in the food chain, responses can be formed to any possible negative situation in *Daphnia* at the ecosystem level (Flaherty and Dodson, 2005). Its lifespan increases with the decreasing temperature due to low metabolic activities (Pennak, 1989). The time required for *Daphnia* to mature varies between 6 and 10 days, which is related to their body size (Pennak, 1989). Daphnia has a life cycle consisting of four significant stages: egg, juvenile, adolescent, and adult (Jonczyk and Gilron, 2005).

Our study also attempted to reveal the effects of paper cups on the reproductive and developmental characteristics of *Daphnia magna*. *Daphnia* can change its reproductive modes from parthenogenesis to sexual reproduction in response to environmental stimuli. This reproductive strategy includes two stages: under normal conditions, they produce clonal female offspring through parthenogenesis and, in response to some adverse environmental and biological factors such as shortened daylight hours, low temperature, overpopulation, and nutrient deficiency, they can switch to sexual reproduction and produce male offspring (Hebert, 1978).

Artemia salina is another organism used in our study to assess the possible impacts of paper cups (Figure 1.2).

Figure 1.2. Larvae and adult individuals of *Artemia salina* (Original)

Artemia salina, a zooplanktonic organism, is used as an important test organism for bio-experimental research in ecotoxicology studies in the marine environment (Madhav et al., 2017) due to its ease of culturing, ready availability, low cost, and adaptation to adverse conditions (Soltanian, 2007).

The main purpose of using these organisms in experimental research is their suitability for laboratory conditions, ease of control, reliability of the obtained data, and providing/producing economically. *A. salina*, an important zooplanktonic organism, is a type of arthropod living in salty lake waters and salt lakes and is an organism resistant to a wide salinity range (1-200‰ ppt). *A. salina* can grow up to 2 cm in its natural environment (Ateş et al., 2013). During growth, *A. salina* molts several times and reaches the adult stage in 12-15 days. The genus *Artemia* has both reproductive and parthenogenic species (Browne, 1980). Under adverse conditions, offspring are produced from cysts that can be kept dormant by washing with salty water even after a few years (Lavens and Sorgeloos, 2000). *A. salina* is utilized as a potential food source (Léger et al., 1986), and its use is recommended by the OECD (Organization for Economic Co-operation and Development) in feeding juvenile fish (OECD-210, 1992). *A. salina*, one of the primary consumer organisms, may be transmitted by fish and crustaceans in the upper consumer group through nutritional (trophic) transfer. Likewise, possible NP exposure and accumulation in *A. salina* may cause an ecological imbalance in the food chain.

Daphnia magna and *Artemia salina* are organisms used to detect the toxicity of some food additives. The effect of activated carbon, whose use in foods has increased considerably in recent years, has been determined in these living things (Fidan and Ayar, 2020).

The present study investigated the possible effects of paper cups of different commercially available brands on *Daphnia magna*, a freshwater organism, and *Artemia salina*, a saltwater organism.

2. Materials and Methods

Paper cups, plastic cups, and glass cups utilized in the study were obtained from a commercial company (Figure 2.1). *Daphnia magna* and *Artemia salina* eggs to be used to assess the effects of paper cups were obtained from a commercial company and cultured in the school laboratory. These cultured organisms are kept alive under standard living conditions for 5 years. *D. magna* was kept in a 120 L aquarium at a temperature of 16–18 ◦C (± 1) and 16 hours of light and 8 hours of dark for one month to ensure its adaptation to acclimatized laboratory conditions. The daily mortality rate was found to be less than 10% during *D. magna* adaptation. *D. magna* was fed once daily with a mixture of dry spirulina powder and baker's yeast (*Saccharomyces cerevisiae*) periodically, and the aquarium water was regularly aerated with an air pump. Furthermore, 25% of the water was renewed at a rate of 1/7 (Figure 2.1).

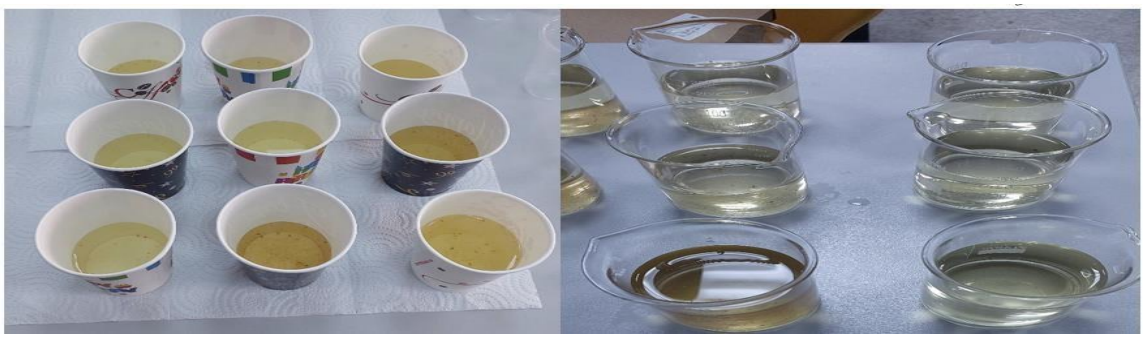

Figure 2.1. Experimental setup prepared to assess mortality

A. salina, among the primary consumer zooplankton species living in the seawater environment, was acquired from a commercial company for the bioassay. *A. salina* eggs were incubated in seawater, and the larvae were hatched. To this end, seawater (30 ‰ m/v: 30 g of synthetic sea salt in 1 Lt water) was prepared by dissolving the synthetic sea salt Instant Ocean® in deionized water in the appropriate amount in the laboratory environment. *A. salina* eggs were hatched or incubated according to the method outlined below (Ates et al., 2013), and the organisms were prepared for the bioassay. About 1 g of pre-cleaned *A. salina* eggs were incubated in a conical plastic graduated container in 1 L of seawater at 30 ± 1 °C. Since *A. salina* eggs hatch in basic environments, the pH level was adjusted to be above 7.6. Ambient lighting was provided with a fluorescent lamp with 1500 lux of daylight. Furthermore, to ensure strong and continuous aeration, the air was supplied to the seawater environment with the help of the aquarium air pump, with the aeration hose extending to the bottom of the hatching system (conical plastic), and the circulation of *A. salina* eggs in the water was provided. Under the said conditions, *A. salina* eggs were mixed for 24-36 hours, and then the newly hatched live offspring were filtered through 30 µm cellulose filters. To be used in mortality studies, 350 mL of culture water at 20 °C was filled into three glass cups, three paper cups, and one plastic cup. Ten first-stage juvenile *Daphnia* and *Artemia* individuals were collected with a pipette into all cups and gently transferred to these cups. The temperature in each cup was adjusted to 20 ◦C (± 1). Changes in water temperature were regularly checked. The test organisms were not fed during the experiment, and the 16:8 hour light: dark photoperiod was maintained. Three replicates were made for each experimental group. The number of dead water fleas and *Artemia* in each cup was counted after 24, 48, 72, and 96 hours. Mortality rates were calculated as a percentage in each experimental group at the end of the test period (96 hours) (Babu et al., 2015).

In the second stage of the study, the water to be used for *Daphnia* and *Artemia* cultures was heated up to 80 °C and put into paper, plastic, and glass cups to reveal the effects of paper cups exposed to high temperatures. After the water temperature in the cups exposed to high-temperature water decreased to 20 °C, the tests were conducted in line with the same test procedure. In this way, the possible effects of the changes that may occur on the inner surfaces of the paper cups from which we drink tea, coffee, etc. in our daily life can be observed. Our study also investigated the effects of paper cups exposed to different temperatures on the developmental and reproductive characteristics of *Daphnia* and *Artemia* individuals (Figure 2.2). Especially body size, the number of eggs produced, and first incubation times were determined and compared.

Figure 2.2. Hatch formation and imaging of eggs in *Daphnia magna* (on the left) and *Artemia salina* (on the right) individuals (original)

3. Results

The survival rates resulting from exposing paper cups of three brands sold in the market to water at different temperatures are presented below (Tables 3.1 and 3.2).

Table 3.1. Survival rates of *Daphnia magna* individuals kept in different habitats (20 °C)

	C.G	**1st P.C.**	**2nd P.C.**	**3rd P.C.**	**1st G.C.**	**2nd G.C.**	**3rd G.C.**
A. salina (n)	10	10	10	10	10	10	10
24 hours	10	10	10	8	10	10	9
48 hours	10	8	9	7	10	8	9
72 hours	10	7	6	7	9	8	9
96 hours	8	6	5	5	9	8	9

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup

Table 3.2. Survival rates of *Artemia salina* individuals kept in different habitats (20 °C)

	C.G	**1st P.C.**	**2nd P.C.**	**3rd P.C.**	**1st G.C.**	**2nd G.C.**	**3rd G.C.**
A. salina (n)	10	10	10	10	10	10	10
24 hours	9	10	9	9	10	9	10
48 hours	9	9	8	8	10	9	10
72 hours	8	7	6	7	9	8	9
96 hours	8	6	6	5	9	8	9

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup

In the study, the survival rates of *Daphnia magna* and *Artemia salina* in paper cups exposed to water at 80 °C, the average temperature of hot beverages, such as tea and coffee, for 10 minutes are shown below (Tables 3.3 and 3.4).

Table 3.3. Survival rates of *Daphnia magna* individuals exposed to water at 80 °C for 10 minutes and kept in cooled cups

	C.G	**1st P.C.**	**2nd P.C.**	**3rd P.C.**	**1st G.C.**	**2nd G.C.**	**3rd G.C.**
D. magna (n)	10	10	10	10	10	10	10
24 hours	10	10	8	9	10	9	10
48 hours	9	9	7	7	10	9	10
72 hours	8	8	6	4	9	9	9
96 hours	8	6	3	4	9	8	9

C.G.: Control group, P.C.: Paper cup, G.C. Glass cup

Table 3.4. Survival rates of *Artemia salina* individuals exposed to water at 80 °C for 10 minutes and kept in cooled cups

	C.G.	**1st P.C.**	**2nd P.C.**	**3rd P.C.**	**1st G.C.**	**2nd G.C.**	**3rd G.C.**
A. salina (n)	10	10	10	10	10	10	10
24 hours	9	9	10	8	10	10	10
48 hours	9	6	8	7	9	10	9
72 hours	8	5	5	6	9	10	9
96 hours	8	3	2	2	8	10	8

C.G.: Control group, P.C.: Paper cup, G.C. Glass cup

The developmental and reproductive properties of *Daphnia* and *Artemia* individuals kept in paper cups exposed to water at 20°C are given below (Tables 3.5 and 3.6).

Table 3.5. Reproductive and developmental characteristics of *Daphnia magna* individuals kept in cups exposed to water at 20 °C for 21 days

	First incubation time (g) ±S.E.	**Number of the days first eggs were obtained (g) ±S.E.**	**Number of the first eggs produced (n) ±S.E.**	**Body length (mm)±S.E.**
C.G. (Beaker)	6±0.67	7±0.77	22±3.92	3.87±0.4
G.C.(1)	5±0.88	6±0.73	23±3.11	3.83±0.2
G.C. (2)	6±1.11	7±1.22	21±2.77	3.88±0.4
G.C. (3)	6±0.44	8±1.12	22±2.56	3.79±0.2
P.C. (1)	8±0.99	9±0.33	14±3.44	3.11±0.6
P.C. (2)	9±1.33	12±0.44	11±2.77	2.98±0.7
P.C. (3)	9±0.77	11±0.33	17±1.88	3.22±0.6

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup, S.E.: Standard error

Table 3.6. Reproductive and developmental characteristics of *Artemia salina* individuals kept in cups exposed to water at 20 °C for 21 days

	First incubation time (g) ±S.E.	**Number of the days first eggs were obtained (g) ±S.E.**	**Number of the first eggs produced (n) ±S.E.**	**Body length(mm)±S.E.**
C.G.(Beaker)	9±0.17	7±0.77	29±3.92	9.87±0.03
G.C.(1)	11±0.88	11±0.88	29±3.11	7.83±0.02
G.C. (2)	10±1.11	13±1.33	28±2.77	8.88±0.01
G.C. (3)	9±0.44	9±2.12	26±2.56	8.79±0.32
P.C. (1)	15±0.99	17±1.77	11±0.33	7.11±0.06
P.C. (2)	14±0.33	15±1.33	13±1.77	5.98±0.33
P.C. (3)	14±0.79	16±2.77	14±2.77	6.22±0.03

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup, S.E.: Standard error

The number of eggs identified in adults of *Artemia salina* individuals was determined by examining *Artemia salina* under a stereo microscope (Figure 3.1).

Figure 3.1. Number of eggs identified in *Artemia salina* adult individuals

The developmental and reproductive characteristics of *Daphnia* and *Artemia* individuals kept in paper cups exposed to water at 80 °C are shown below (Tables 3.7 and 3.8).

Table 3.7. Reproductive and developmental characteristics of *Daphnia magna* individuals kept in cups exposed to water at 80 °C for 21 days

	First incubation time (g) ±S.E.	**Number of the days first eggs were obtained (g) ±S.E.**	**Number of the first eggs produced (n) ±S.E.**	**Body length (mm)±S.E.**
C.G.(Beaker)	5±0.33	8±0.77	23±3.11	3.77±0.04
G.C.(1)	5±0.77	7±0.73	24±0.33	3.55±0.02
G.C. (2)	6±1.22	7±1.22	22±1.77	3.58±0.04
G.C. (3)	5±0.44	9±1.12	23±2.56	3.79±0.32
P.C. (1)	12±0.99	11±0.33	14±3.44	2.01±0.06
P.C. (2)	11±1.33	13±0.44	14±2.77	288±0.07
P.C. (3)	13±0.77	12±0.33	15±1.88	3.01±0.06

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup, S.E.: Standard error

Table 3.8. Reproductive and developmental characteristics of *Artemia salina* individuals kept in cups exposed to water at 80 ºC for 21 days

	First incubation time (g) ±S.E.	**Number of the days first eggs were obtained (g) ±S.E.**	**Number of the first eggs produced (n) ±S.E.**	**Body length(mm)± S.E.**
C.G.(Beaker)	8±0.33	8±0.77	29±3.11	9.87±0.04
G.C.(1)	9±0.77	7±0.33	24±0.33	9.83±0.02
G.C. (2)	9±1.22	8±1.33	22±1.77	8.88±0.04
G.C. (3)	7±0.44	9±1.22	23±2.56	7.79±0.77
P.C. (1)	17±0.33	19±0.33	9±3.44	4.12±0.03
P.C. (2)	16±1.77	17±0.44	8±2.77	5.33±0.33
P.C. (3)	16±1.22	17±0.33	11±1.88	4.98±0.06

C.G.: Control group, P.C.: Paper cup, G.C.: Glass cup, S.E.: Standard error

To determine the reproductive characteristics of *Artemia salina* and *Daphnia magna* individuals, the first incubation period and the number of days the first eggs were obtained were determined by observing all individuals under a stereo microscope for 21 days (Figure 3.2).

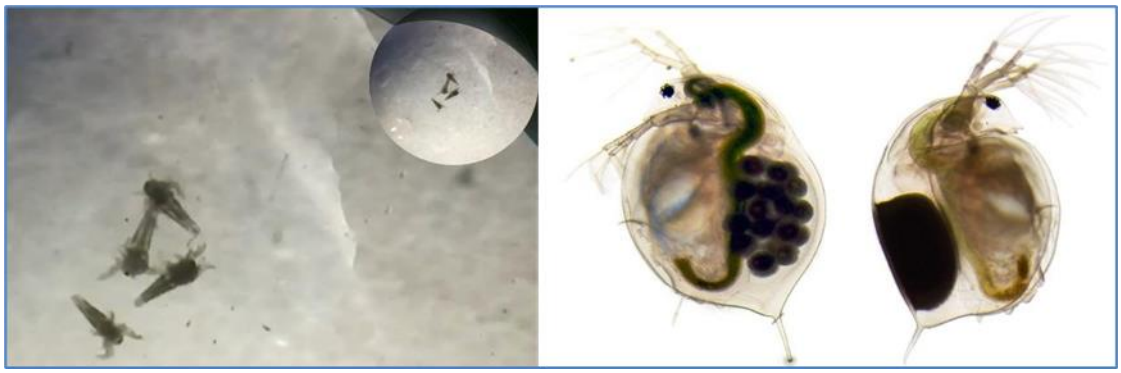

Figure 3.2. Detection stages of the incubation period and first egg production day of *Artemia salina* and *Daphnia magna*

4. Conclusion and Discussion

Two different tests were conducted on two different organisms to observe the effects of different types of paper cups in the study. While the first test was carried out in cups exposed to water at 20 °C, the standard living conditions of living beings, the second was performed in cups exposed to 80 °C water.

As seen in Table 3.1, the survival rates of *Daphnia magna* individuals kept in glass cups and glass Beaker environments exposed to 20 °C were around 80% and 90%. In the study, while 80% of the individuals kept in the Beaker environment used as the control group survived at the end of 96 hours, the survival rates in different paper cups were determined as 50% and 60%. The survival rates obtained in three different paper cups were lower compared to the glass cup and control group (Beaker) individuals. In Table 3.2, the survival rates of *Artemia salina* individuals kept in glass cups and glass Beaker environments exposed to 20 °C temperature were determined as 80% in the control group. In individuals kept in a glass cup environment, the survival rate was found to be 90% in two of them and 80% in one of them. Considering *Artemia* individuals kept in a paper cup environment, the survival rate in two of them was 60% at the end of 96 hours, while it was determined as 50% in one of them.

As seen in Table 3.3, the survival rate of *Daphnia magna* individuals, which were exposed to water at 80 °C for 10 minutes and then kept in environments that were expected to cool, decreased to 30% in paper cups at the end of 96 hours. The survival rate was found to be 80% and 90% in the individuals kept in glass cups and the glass Beaker environment used as the control group. Upon examining Table 3.4, it was observed that the survival rate of *Artemia salina* individuals, which were exposed to water at 80 °C for 10 minutes and then kept in environments that were expected to cool, decreased to 20% in paper cups at the end of 96 hours. The values acquired again were lower than the control group and glass cup environments in the comparison made with three different brands of paper cups.

As seen in Table 3.5, some developmental and reproductive characteristics of *Daphnia magna* individuals exposed to water at 20 °C for 21 days were examined. Whereas the first incubation period was 6 days in the control group individuals, this period extended up to 9 days in the individuals kept in paper cup environments. An increase was observed in the number of days when the first eggs were obtained in the paper cup environment in comparison with the control group and the glass cup environment. Considering the body sizes, while the average value was 3.8 mm in the control group, the mentioned value decreased to 2.98 mm in the individuals kept in the paper cup environment.

As seen in Table 3.6, some developmental and reproductive characteristics of *Artemia salina* individuals exposed to water at 20 °C for 21 days were examined. An extension was detected in the first incubation period and the number of days the first eggs were obtained n individuals kept in the paper cup environment compared to the control group and glass cup environment. There was a decrease in the number of eggs obtained and body size in individuals kept in the paper cup environment. In the parameters observed in Tables 3.7 and 3.8, where the effects of the water environment with a temperature of 80 °C on paper cups were investigated, it was observed that developmental characteristics were impacted more adversely, and likewise, reproductive characteristics were negatively impacted. For example, as seen in Table 3.7, the body sizes of *Daphnia magna* decreased further. Similarly, it was seen that a more significant decrease occurred in body size in *Artemia salina* individuals than in individuals kept in environments exposed to water with a temperature of 20 °C.

Considering the results acquired in our study, it is seen that paper cups had more negative effects than the control group and glass cups under both temperature conditions (20 °C and 80 °C). It was revealed that adverse effects were higher in both organisms, particularly in cups exposed to water at 80 °C. We think that the hydrophobic film layer on the inner surface of paper cups, which deteriorates when it contacts hot water, is effective in this result.

Different studies with paper cups determined that when these cups were exposed to water and especially when they were exposed to high-temperature water, some microplastics and some different substances were released into the water environment. For example, the study by Ranjan et al. (2021) found that paper cups exposed to water at 85-90 degrees for 15 minutes released microplastic particles into the water. Plastic particles with the size of 25000 microns were detected in fluorescent microscopic imaging. Toxic heavy metals, such as Pb, Cr, and Cd, were detected in electron micrographic imaging.

Additionally, it was determined that paper cups were made of 90-95% paper, but 5% were covered with a hydrophobic film on their inner surfaces, and this layer was made using polyethylene and copolymer alternatives (Sabit, 2016). As a result, it is observed that the use of paper cups, preferred due to their ease of use and practicality, carries risks, especially with hot drinks. Whereas the polyethylene layer on the inner surface of the cup degrades less at normal temperatures, this impact gradually increases at high temperatures. Hence, the use of glass cups emerges as the most appropriate option when consuming these types of beverages.

5. Recommendations

Disposable paper cups are widely used in areas where tea and coffee are sold worldwide. Moreover, they are frequently used due to their affordable price, lightweight, and acting as a barrier to moisture and oxygen. However, recent studies also demonstrate that there may be some changes in the inner surface of paper cups, depending on the increase in temperature. The fact that this situation increases due to the increasing temperature indicates that these cups may be suitable for consuming non-hot beverages. Nevertheless, some negative consequences may occur for human health, particularly at temperatures of 80 °C and above. The fact that this effect does not emerge in a short time for users does not hurt these cups. However, it can be observed especially in studies conducted with model organisms. Hence, to raise people's awareness, the number of such studies should be increased, and the effect should be shared with the public.

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Review

An overview of environmental attitudes, awareness, sensitivity, and literacy of nursing students in Turkey

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Abstract

The environmental factors are vital determinants for health and wellness of organisms. Because of the population increase, technological developments, more industrial activities and dense traffic, environmental pollution, and destruction became one of the major problems in the world. Various studies indicated that most of the diseases and health problems are closely related with negative environmental factors. Considering the roles and responsibilities of the nurses, increasing the knowledge and awareness of nursing students on environmental issues has vital importance in terms of protecting and improving health. This paper aimed to evaluate the studies on environmental attitudes, awareness, sensitivity, and literacy of nursing students in Turkey. This assessment is important to improve and upgrade these characteristics of nursing students and nurses. It also has an importance for public health. The results of the studies generally indicated that majority of the nursing students had moderate levels of environmental attitudes, awareness, sensitivity, and literacy. Most of the studies confirm that the levels of nursing students on these subjects closely related with components of nursing education and content and composition of subjects. The common result obtained from the studies is to increase the number of subjects and social activities on environmental issues in nursing education for higher environmental awareness, sensitivity, and literacy of nursing students, understanding, and eliminating the problems arising from environmental factors. Considering the roles and responsibilities of the nurses, increasing the knowledge and awareness of students on environmental issues has vital importance in terms of protecting and improving health.

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1. Introduction

Organisms, which are the biotic components of ecosystems, continuously interact with their environment. Environmental factors affect organisms biologically, physically, chemically,

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and socially, in different ways. Therefore, environmental factors are vital for health and wellness of organisms. Climate conditions, soil, water, and air status in an environment are basic abiotic factors that are effective on organisms.

In recent years, technological developments, increase in population, industrial activities and traffic lead to environmental pollution and destruction. Environmental pollution and destruction alter natural habitat conditions of organisms. These differences significantly affect metabolism of living organisms and cause various damages and disease.

Humans need many resources to live healthy. They interact with biotic and abiotic factors of the ecosystem for food, water, respiration, shelter, and other activities. Changes in environmental factors may be harmful directly or indirectly for human health. A differences or damage in an environmental factor may negatively affect human life in several ways. Contaminated food, water and air, limited nutrient, hazardous substances, vector organisms, harmful microorganisms, and habitat disturbance cause several problems such as disease and stress. Various studies indicated that several diseases and health problems are closely related with negative environmental factors.

So, understanding these relationships is vital for health and wellness of humans. It is known that anthropogenic activities are major impacts of environmental problems. The important point is to prevent these activities before environmental problems occur. Because of this, it is important to have knowledge about the factors that threaten the environment and human health.

Environmental health is an important component of public health (Polivka et al., 2012; Cruz et al., 2018). Because of their responsibilities in health protection and promotion, nurses' knowledge and awareness of environmental issues is very important for public health.

This study aimed to discuss the environmental awareness of nursing students in Turkey by evaluating the previous studies. There are several studies on environmental attitudes, awareness, sensitivity, and literacy of nursing students both in the world (Wright, 2003; Camacho RodrÝg uez and Jaimes Carvajal, 2016; Cruz et al., 2018; Felicilda-Reynaldo et al., 2018; Ha and Lee, 2019; Anåker et al., 2021; Álvarez-Nieto et al., 2022 a,b) and Turkey (Çınar et al., 2010; Bodur and Taşocak, 2013; Çelik, 2016; Sayan and Kaya, 2016; Beser et al., 2017; Baybuga and Sönmez, 2019; Bakan et al., 2020; Mercan and Mercan, 2020; Ergin et al., 2021; Gök and Kılıç, 2021; Kapan and Gürel, 2022; Örs, 2022). By these studies, status of

the knowledge of nursing students on environmental attitudes, awareness, and literacy were evaluated.

2. Evaluation of studies on nursing students in terms of environmental issues in Turkey

Some of the studies conducted to evaluate the status of nursing students about the environmental issues in Turkey were summarized and presented in the Table 1. Various scales were used to monitor environmental attitudes, awareness, sensitivity, and literacy of nursing students in these studies. Almost, most of the studies reported moderate environmental attitudes, awareness, sensitivity, and literacy levels for nursing students.

Attitudes of nursing students about environmental problems were studied by Çınar et al., (2010), Karahan Okuroğlu (2012), Çelik et al., (2016), Sayan and Kaya (2016), Beser et al., (2017), Baybuga and Sönmez (2019), Bakan et al. (2020). In the study of Çınar et al. (2010), "Environmental Attitude Scale", which was prepared by Berberoğlu and Tosunoğlu (1995), applied to all senior class students of Nursing Department in Sakarya University. It was determined that while the attitudes of nursing students towards environmental problems in many subjects were close to the desired levels, some subjects considered as environmental risks were not perceived as risky enough by the students. According to the results, there was not a significant relationship between the scores and parental education, gender, economic status, and place of residence. Karahan Okuroğlu (2012) applied the “Ecocentric, Anthropocentric and Antipathetic Attitudes towards Environment Scale” to the graduate nursing students at Istanbul University. The fourth-class students' ecocentered attitude score was clearly higher than the second-class students, and their antipathetic attitude score towards the environment was lower than the first-class students and second-class students. Additionally, the mean ecocentric attitude of the female students was higher than male students. No significant relationship was found between the educational status of the parents, income level of the family, longest place of residence unit of the students, being a member of any environmental organization and the mean scores of ecocentric, anthropocentric and antipathetic attitudes to the environment.

Çelik et al. (2016) reported that the nursing students are less sensitive to the environmental issues than the medical faculty students. The demographic characteristics were effective on students’ attitudes to the environmental issues.

Sayan and Kaya (2016) studied the environmental attitudes and the environmental risk perceptions of the nursing students. Significant moderate correlation was determined between these factors. Additionally, it was found that some characteristics of the students such as gender, partipication to the environmental organizations and interest to the environmental subjects were effective on perception of environmental risks and environmental attitudes.

Beser et al. (2017) and Bakan et al. (2020) aimed to determine the attitudes of nursing students towards environmental problems. Higher scores were determined for fourth year nursing students and becoming a member of environmental organizations increased the scores by Beser et al. (2017). Similarly, Bakan et al. (2020) reported high positive environmental attitude scores for nursing students. Baybuga and Sönmez (2019) conducted a descriptive study on nursing students' environment-centered, human-centered and antipathetic attitudes towards environment. The attitudes of nursing students to the environmental issues were positive. The scores of ecocentric and anthropocentric attitudes of the students were high while their antipathetic attitude score was low.

Ulaş Karaahmetoğlu (2017) compared faculty of forestry students and nursing students in terms of environmental sensivity and found that nursing students have less environmental sensitivity than faculty of forestry students. This result showed that education is very effective on environmental sensitivity. It can be said that environmental awareness increases as the level of knowledge about the environment increases.

Tunçay (2019) explained that environmental ethics awareness levels of the students were high.

Taking education on this subject had positive effects on awareness scores of students. It was found that there was not any variation in environmental ethic levels among students according to gender, age, class etc.

Gök and Kılıç (2021) reported moderate levels of environmental sensitivity and awareness for nursing studens in their study. They explained that social activities and lectures on the environmental issues may improve environmental awareness levels. Similar results were determined in the studies of Ergin et al. (2021), Uzelli Yılmaz (2021), Örs (2022), Kapan and Gürel (2022). These were generally reported moderate environmental literacy levels.

According to results of the studies, environmental knowledge of nursing students is weak in terms of specific environmental issues. They usually had general information about ecological

factors and environmental processes. It is understood that they need more detailed information and experience to understand the requirements of environmental health.

Table 1. Some of the studies on environmental attitudes, awareness, sensitivity, and literacy of nursing students in Turkey

Author	Subject	Result
Çınar et al., (2010)	Department of Senior Nursing Students' Attitudes Toward Environmental Problems	Attitudes towards environmental subjects in many subjects close to the desired level; some environmental risk is not perceived as risky
Karahan Okuroğlu, (2012)	The impact of nursing education on Students' attitudes towards the environment	High environment-centered attitude mean score; low indifferent attitude scores towards the environment
Bodur and Taşocak, (2013)	Nursing students' views about environmental sensitivity in Turkey	Medium-level sensitivity toward environment
Çelik et al., (2016)	Nursing Department and Medical Faculty Students' Attitudes towards Environmental Problems	High environmental awareness: demographic factors affect environmental attitudes
Ulaş Karaahmetoğlu et al., (2017)	Analysis of nursing and faculty of forestry students' views on environmental sensitivity	Students of faculty of forestry has more environmental sensitivity than students of nursing.
Sayan and Kaya, (2016)	Assessment of the environmental risk perceptions and environmental attitudes of nursing students	Scored highly on environmental risk perceptions
Beser et al., (2017)	Examination of nursing students' attitudes towards environmental problems.	Fourth year students had higher scores for their attitudes to environmental problems
Baybuga and Sönmez, (2019)	Turkish nursing students' ecocentric, anthropocentric and antipathetic attitudes towards the environment	High average ecocentric attitude score, an average anthropocentric attitude score and a low average antipathetic attitude score
Tunçay, (2019)	The Nurse Candidates' Awareness Levels to Environmental Ethics (Specific Çankırı Karatekin University)	High awareness levels to environmental ethics
Bakan et al., (2020)	University students' attitudes towards environmental problems	Positive attitudes towards environmental problems, 67.3% of the students have high scores

Ergin et al., (2021)	A mixed method study on global warming, climate change and the role of public health nurses from the perspective of nursing students	Adequate awareness and knowledge level about global warming and climate change
Gök and Kılıç, (2021).	Environmental awareness and sensitivity of nursing students	Moderate environmental awareness and sensitivity
Örs, (2022)	A measurement of the environmental literacy of nursing students for a sustainable environment	High score for the use and concern dimensions of environmental literacy, and moderate attitudes towards the environment, low scores for the environmental knowledge
Uzelli Yılmaz et al. (2021)	Determination of awareness levels of a group of nursing students towards environmental ethics	High awareness levels of environmental ethics
Kapan and Gürel (2022).	An evaluation of the environmental literacy levels of nursing students in Turkey	Moderate environmental literacy levels

3. Conclusions

According to the studies, the levels of the environmental attitudes, awareness, sensitivity, and literacy of nursing students in Turkey are moderate. The levels of nursing students on these subjects closely related with components of education and composition of subjects. Most of the studies confirm and agree with this opinion. These results showed that environmental subjects in nursing education are insufficient. Today, environmental issues are within the basic vital problems in the world. Problems such as healthy food, water supply, air, soil, water pollution, global climate change are important factors that threaten humanity and our world. Considering the roles and responsibilities of the nurses, increasing the knowledge and awareness of students on environmental issues has vital importance in terms of protecting and improving health. As the number of studies showing the negative effects of environmental problems on human health increases, the necessity of this is better revealed. In this context, the common result obtained from the studies is to increase the number of subjects and social activities on environmental issues in nursing education for higher environmental awareness, sensitivity, and literacy of nursing students, understanding, and eliminating the problems arising from environmental factors. For this purpose, students should be supported with social

responsibility projects, seminars, and courses. People with a high level of environmental knowledge are also better at environmental protection. Since nursing is a profession that is a role model for the society, it is very important for public health to bring this attitude to nurses. When today's conditions are evaluated ecologically in terms of public health, it has been concluded that students should be more directed to environmental health nursing. Preventing diseases caused by environmental problems and living in a clean environment are very important in the formation of a healthy society.

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