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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.

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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).

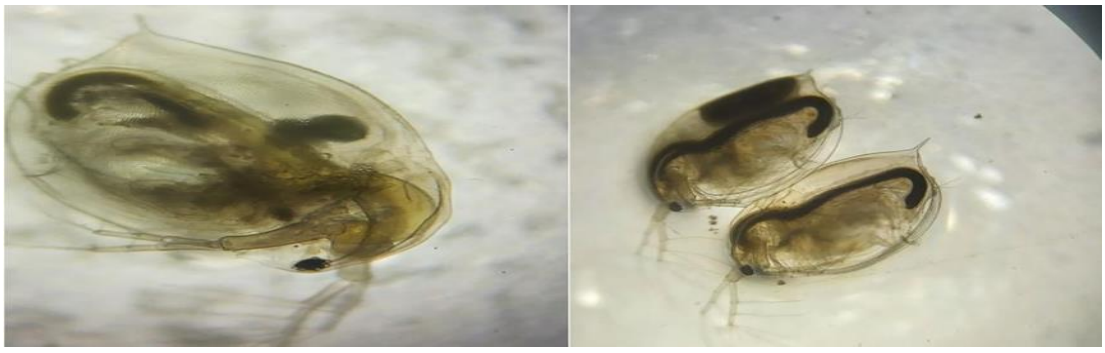

Figure 1.1. *Daphnia magna* (Original)

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).

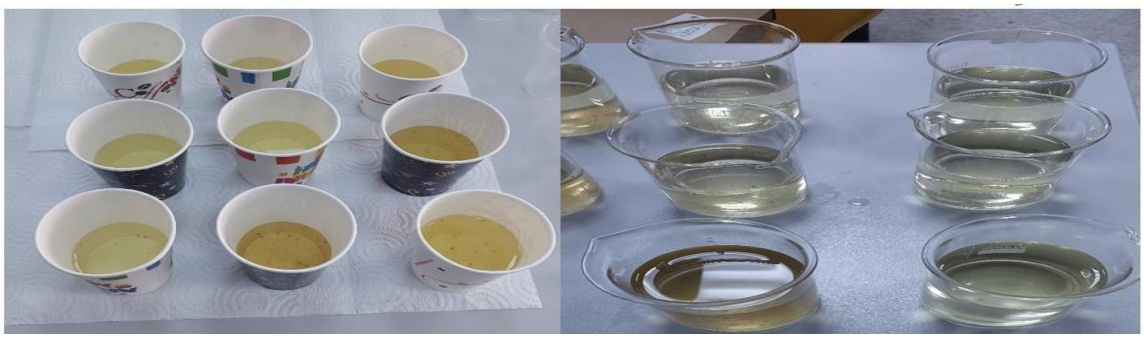

Figure 2.1. Experimental setup prepared to assess mortality

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).

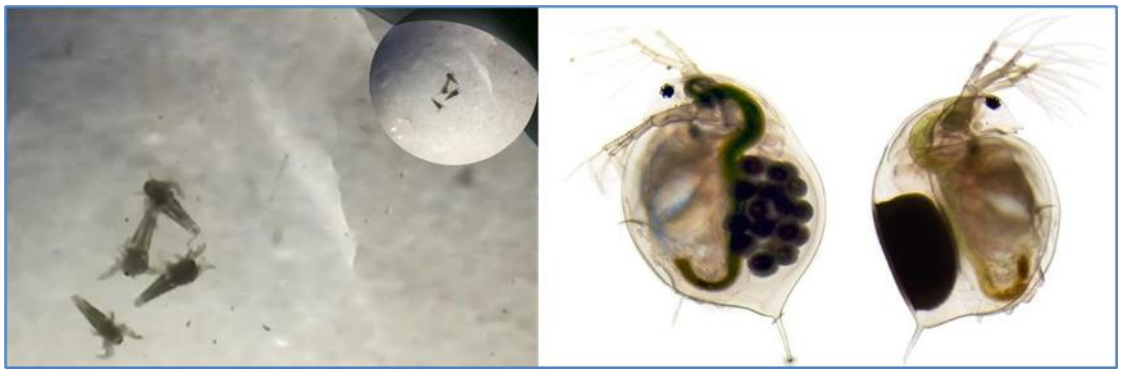

Figure 3.2. Detection stages of the incubation period and first egg production day of *Artemia salina* and *Daphnia magna*

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.

References

Ahmed, R., Hamid, A.K., Krebsbach, S.A., He, J., Wang, D. (2022). Critical review of microplastics removal from the environment. Chemosphere, 293.

Ateş, M., Daniels, J., Arslan, Z., Farah, I.O. (2013). Effects of aqueous suspensions of titanium dioxide nanoparticles on *Artemia salina*: assessment of nanoparticle aggregation, accumulation,and toxicity. Environmental Monitoring and Assessment, 185:3339–3348.

Akçay, S., Törnük, F., Yetim, H. (2020). Mikroplastikler: Gıdalarda Bulunuşu ve Sağlık Üzerine Etkileri [Microplastics: Effects on Health and Occurrence in Foods]. Avrupa Bilim ve Teknoloji Dergisi, (20), 530-538.

Arumugam, K., Renganathan, S., Babalola, O. O., & Muthunarayanan, V. (2018). Investigation on paper cup waste degradation by bacterial consortium and Eudrillus eugeinea through vermicomposting. Waste Management, 74,185–193.

Aydın, S., Ulvi, A., Aydın, M, A., Ulvi, A. (2019). Mikroplastikler ve çevresel etkileri. 4. Uluslararası Bilimsel Araştırmalar Kongresi [Microplastics and their environmental effects. 4th International Scientific Research Congress.]. February 14-17, Ankara: Doğuhan Yayıncılık, 17- 26.

Browne, R.A. (1980). Competition experiments between parthenogenetic and sexual strains of the brine shrimp, *A. salina*. Ecology, 61:471–474.

Elmas, Gulnur Mertoglu Çınar, G. (2018). Toxic Metals in Paper and Paperboard FoodPackagings. BioResources, 13(4), 7560-7580.

Esmeray, E., Armutçu, C. (2020). Microplastics, Environmental-Human Health Effects and Analysis Methods. Düzce University Journal of Science and Technology, 8, 839-868.

Fidan, M. & Ayar, A. (2020). An in vivo study on *Drosophila melanogaster*, *Artemia salina*, and *Daphnia magna*: Is activated carbon used as a food additive reliable? . International Journal of Science Letters , 2 (2) , 79-91 .

Flaherty, C. M., and Dodson, S. I. (2005). Effects of pharmaceuticals on Daphnia survival, growth, and reproduction. Chemosphere, 61(2), 200-207.

Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., & Purnell, P. (2018). An overview ofchemical additives present in plastics: Migration, release, fate and environmental impact duringtheir use, disposal and recycling. Journal of Hazardous Materials, 344, 179–199.

Hernandez, L. M., Xu, E. G., Larsson, H. C. E., Tahara, R., Maisuria, V. B., & Tufenkji, N. (2019). Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea. Environmental Science and Technology, 53(21), 12300–12310.

Internet: http://learn.genetics.utah.edu/content/gsl/artemia/ (Last access date:14/07/2022).

Kutralam-Muniasamy, G., Pérez-Guevara, F., Elizalde-Martínez, I., & Shruti, V. C. (2020). Branded milks – Are they immune from microplastics contamination? Science of The Total Environment, 714, 136823.

Lavens, P., Sorgeloos, P. (2000). The history, present status and prospects of the availability of

A. salina cysts for aquaculture. Aquaculture, 181:397–403.

Léger, P., Bengtson, D., Simpson, K., Sorgeloos, P., 1986. The use and nutritional value of A.salina as a food source. Oceanogr. Annual Review of Marine Science, 24:521–623.

Li, S., and Tan, Y. (2011). Hormetic response of cholinesterase rom *D. magna* in chronic exposure to triazophos and chlorpyrifos. Journal of Environmental Sciences, 23(5), 852-859.

Madhav, M.R., David, S.E.M., Kumar, R.S., Swathy, J.S., Bhuvaneshwari, M., Mukherjee, A., Chandrasekaran, N. (2017). Toxicity and accumulation of Copper oxide (CuO) nanoparticles in different life stages of A. salina. Environmental Toxicology and Pharmacology, 52: 227– 238.

Pennak, R.W., 1989, Fresh-water invertebrates of the United States, John Wiley & Sons, NewYork, 628p.

Ranjan, V. P., Joseph, A., & Goel, S. (2021). Microplastics and other harmful substances released from disposable paper cups into hot water. *Journal of Hazardous Materials*, *404*, 124118.

Rogovina, S., Aleksanyan, K., Prut, E., & Gorenberg, A. (2013). Biodegradable blends of cellulose with synthetic polymers and some other polysaccharides. European Polymer Journal,49(1), 194–202.

Sathish, M. N., Jeyasanta, I., & Patterson, J. (2020b). Occurrence of microplastics in epipelagicand mesopelagic fishes from Tuticorin, Southeast coast of India. Science of The Total Environment, 720, 137614.

Schultes, L., Peaslee, G. F., Brockman, J. D., Majumdar, A., McGuinness, S. R., Wilkinson, J.T., Sandblom, O., Ngwenyama, R. A., & Benskin, J. P. (2019). Total Fluorine Measurements in Food Packaging: How Do Current Methods Perform? Environmental Science and Technology Letters, 6(2), 73–78.

Soltanian, S. (2007). Protection of gnotobiotic *A. salina* against Vibrio campbellii using baker'syeast strains and extracts. Phd Thesis Ghent University, Belgium, 239s.

Souza, V. G. L., & Fernando, A. L. (2016). Nanoparticles in food packaging: Biodegradabilityand potential migration to food—A review. Food Packaging and Shelf Life, 8, 63–70.

OECD Organisation for Economic Co-operation and Development, (1992). Guideline No. 210. Fish, Early-Life Stage Toxicity Test (original Guideline, Adopted 17th July 1992). OECD, Paris, France.

Tatarazako, N., and Oda, S. (2007). The water flea *D. magna* (Crustacea, Cladocera) as a test species for screening and evaluation of chemicals with endocrine disrupting effects on crustaceans. Ecotoxicology, 16(1), 197-203.

Jonczyk, E., and Gilron, G. U. Y. (2005). Acute and chronic toxicity testing with *Daphnia* sp. InSmall-scale fresh water toxicity investigations (pp. 337-393). Springer, Dordrecht.

Vandermarken, T., Boonen, I., Gryspeirt, C., Croes, K., Van Den Houwe, K., Denison, M. S., Gao, Y., Van Hoeck, E., & Elskens, M. (2019). Assessment of estrogenic compounds in paperboard for dry food packaging with the ERE-CALUX bioassay. Chemosphere, 221, 99– 106.

Virsek, M, K., Palatinus, A., Koren, S., Peterlin, M., Horvat, M., Krzan, A. (2016). Protocol for Microplastics Sampling on the Sea Surface and Sample Analysis. J. Vis. Exp., 118, 1-9.

Xue, M., Chai, X.-S., Li, X., & Chen, R. (2019). Migration of organic contaminants into dry powdered food in paper packaging materials and the influencing factors. Journal of Food Engineering, 262, 75–82.

Yurtsever, M. (2015). Mikroplastiklere Genel Bir Bakış [An Overview of Microplastics]. Dokuz Eylül Üniversitesi MühendislikFakültesi Fen ve Mühendislik Dergisi, 17, 68-83.