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Research Article

An investigation of the toxic effects of water samples collected from 3 different regions of Antarctica on *Drosophila melanogaster*

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Abstract

In this research, it was aimed to investigate the ecotoxicological effects of seawater from Galindez Island, lake sediment samples collected from Ardley Island, and green algae ice samples collected from Horseshoe Island on *Drosophila melanogaster*, which is an important model organism. Newly hatched *Drosophila melanogaster* larvae of the same age and adult individuals were used. While the individuals in the control group were tested in standard media, the individuals in the experimental group were tested under 3 different conditions at the rates of 25%, 50%, and 100% of each water sample. The effects of polar water added to the media on mortality rates on *Drosophila melanogaster* eggs, larvae, and adults were investigated. The effect of water samples collected from Ardley and Horseshoe Islands on the survival percentage in *Drosophila melanogaster* larvae was found to be similar to the control group. Furthermore, while the viability rate in Ardley and Horseshoe Island was 92% and 96%, respectively, in the control group individuals, similar results were obtained in all rates in the experimental group. The water samples obtained from 3 different points from the Antarctic region have not reached a level that will adversely affect the lives of the larvae and adults of the creature as of the present day. Nevertheless, although pollution was detected in some areas in the Antarctic region in the literature, we consider that this pollution can be prevented before it reaches dangerous levels with some measures to be taken.

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Antarctic marine pollution, *Drosophila melanogaster*, Ecotoxicity

1. Introduction

In earlier times, the oceans were considered a reservoir where pollutants can be easily discharged. Organochlorine compounds, domestic and industrial wastes, and petroleum products cause adverse effects in the ocean environment even when they are released at low

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levels (Pinto et al., 2003). As mining and industrial activities increased in the 19th century, environmental pollution caused by organic compounds increased and has also continued to increase since then (Colepicolo et al., 2008).

Different methods have been developed to determine the level of this increasing pollution. The use of marine sediments as markers to detect the presence of environmental pollutants is one of these methods (Ergin et al., 1991). Many toxic and bioaccumulative pollutants accumulate in sediment at higher amounts than in water (Binelli and Provini, 2003). Important organic compounds that accumulate in sediments include PAH (Polycyclic Aromatic Hydrocarbons) and dl-PCB (Dioxin-Like Polychlorinated Biphenyl) compounds (Lacorte et al., 2006). Toxicity caused by organic pollutants leads to an increasing concern due to its bioaccumulation and persistence in the environment (Zhou et al., 2014).

Polar regions are considered clean environments without significant sources of pollution. Especially since Antarctica is far from any urbanization, the pollutant rate of this region is considered to be very low. Antarctica has been considered by humans as an undisturbed region for years (Kim et al., 2006). Nevertheless, global warming, population growth, and industrial development in the Southern Hemisphere countries increase the effects of pollutants on the Antarctic environment (Bargagli, 2008). The main human activities in Antarctica are scientific research and the bases established for this purpose, shipping traffic, waste disposal, fishing, and tourism (Aislabie et al., 1999).

Antarctica is a continent located in the southernmost part of the Southern Hemisphere and includes the South Pole. The Antarctic Peninsula, the only extension of the continent, is also the closest part of Antarctica to South America. It is the only continent without a country. It is the world's driest place in that no rain fell in some parts of the continent for 2 million years. Antarctica is covered by an ice sheet with an average thickness of 2000 m. The ice thickness at the South Pole reaches 4335 m. This ice mass constitutes approximately 90% of all ice on the earth (Kırkıncı et al., 2021).

The Antarctic Peninsula has many research bases since it has the continent's mildest climate. The Antarctic Peninsula consists of a series of rocky islands under the ice sheet that covers it. These rocky islands are connected by an ice sheet that acts as soil (Stewart, 2011). Of Antarctica, 98% is covered with the ice sheet. It has been calculated that if all of this ice sheet, which constitutes approximately 90% of the world's ice and 70% of the freshwater, melts, the sea level will rise by approximately 60 m all over the world (Howstuffworks, 2017).

In polar regions, no pollution is expected since there is no source of pollutants. However, persistent organic pollutants have been encountered in Antarctica since the beginning of the 1960s (Bidlemen et al., 1993). Human activities are among the main causes of pollution in the environment. Human activities in Antarctica began with the establishment of research bases. Subsequently, situations such as increasing research activities, tourism, fishing, shipping and vehicle traffic, and waste disposal have caused pollution in Antarctica (Kim et al., 2006). Air currents are considered one of the most important ways for pollutants to reach Antarctica (Wania and Mackay, 1993). These transported pollutants are trapped on ice or land by precipitation or atmospheric precipitation and begin to accumulate. During the summer months, the pollutants released by the melting of ice and snow mix directly with seawater (Geisz et al., 2008). Different studies are conducted on the determination of pollutants. One of these studies was conducted by Kim et al. (2006). In this study, 621 ng/g – 5024 ng/g T-PAH value was determined in dry weight in the sediment around the McMurdo Research Station. Borghesi et al. (2008) found 15.1 ng/g dl-PCB in dry weight in the liver tissue of *Trematomus bernacchii* organism.

Our study aimed to determine the possible effects of water samples obtained from three different stations in the Antarctic Peninsula on the larvae and adult individuals of *Drosophila melanogaster* individuals.

Drosophila melanogaster, also known as the vinegar fly, is one of the most commonly used model organisms in life sciences. Thomas Hunt Morgan won the Nobel Prize in Physiology and Medicine in 1933 with his study on the chromosomal theory of inheritance using *Drosophila melanogaster* for the first time, showing that the white gene is inherited on the X chromosome (Morgan and Bridges, 1916). Hermann Müller received another Nobel Prize in 1946 with his study investigating the effect of X-rays on mutation rates (Muller, 1928). Based on these discoveries, balancer chromosomes that inhibit recombination through DNA inversion emerged, and these developments showed that mutations were conserved over generations on a single chromosome and made *Drosophila melanogaster* the first genetic system used in genetic research (Lindsley and Zimm, 2012). Successive genetic discoveries allowed for conducting more complex genetic studies. These genetic discoveries in the vinegar fly have made significant contributions to many medical and scientific studies, including gene biology, cell biology, developmental biology, population genetics, molecular genetics, toxicology, and resistance development in insects (Wilson, 1988).

Drosophila melanogaster is used in genetic studies due to its simple genetic structure with 4 pairs of chromosomes. The sequencing of the *Drosophila melanogaster* genome was completed in 2000. Thus, 13600 genes were identified, 95% of which were encoded in 3 of 4 pairs of chromosomes. As a result of the analyses, it was found that different genes associated with human diseases had 77% similarity with *Drosophila melanogaster*. Nevertheless, it was determined that the proteins involved in the regulation of gene expression and metabolism were similar to human genes (Rand, 2010).

2. Materials and Methods

2.1. Materials

2.1.1. Sampling studies

The samples used in the study were provided in 2019 within the scope of the project carried out by Assist. Prof. Yılmaz KAYA and supported in the TAE-III expedition carried out under the auspices of the Presidency, under the responsibility of the Ministry of Industry and Technology, and by the coordination of ITU Polar Research Center. The samples obtained were kept in the cold in the laboratory of the Faculty of Agriculture of 19 Mayıs University until the period of use in our study. The obtained samples were collected from the regions with the coordinate system in Table 1.

Table 1. Effects of seawater sample collected from Galindez Island on *D. melanogaster* eggs, larvae, and adult individuals

No	Region	Coordinate		Sample type
YO	Ardley Island	62º 12' 48,81" S	58º 56' 21,09" S	Lake Sediment
Y13	Galindez Island	65° 14' 41,5" S	64º 15' 22,5" S	Seawater
Y15	Horseshoe Island	67º 49' 42,39" S	67º 13' 29,88" S	Green Algae Ice

In the study, lake sediment, seawater, and green algae ice samples were collected from 3 different stations (Figure 1). Galindez Island, Horshoe Island, and Ardley Island were some of the regions visited by our Turkish scientists to conduct scientific studies within the scope of the National Polar Science Program (2018-2022) carried out under the auspices of the Presidency, under the responsibility of the Ministry of Industry and Technology and the coordination of TÜBİTAK Marmara Research Center (MAM) Polar Research Institute (KARE) (Figure 2).

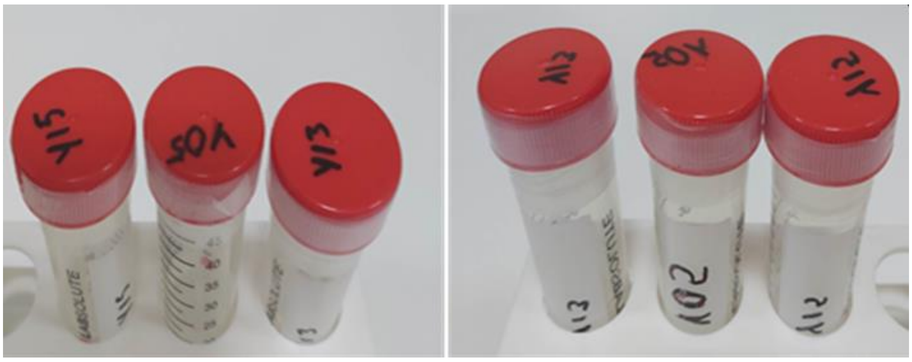

Figure 1. Samples collected from 3 different regions from the Antarctic Peninsula (original)

Figure 2. Islands visited by Turkish scientists to conduct studies (Wikipedia, 2021)

2.1.2. Drosophila melanogaster

The fruit flies to be used in the study were obtained from Amasya University Faculty of Arts and Sciences Biological Research Laboratory. The Oregon (wild type) strain of *D. melanogaster* was used in the experiments. Before the studies as an experimental organism, female and male individuals of the Oregon stock were crossed, and the eggs obtained from this crossing and the larvae and adult individuals obtained from the eggs were used.

The instant *Drosophila* medium was used in the control and experimental groups. The instant medium was prepared using 1.5 grams of the medium and 5 ml of distilled water. The water obtained from the poles, the effect of which was investigated, was added to the medium content by 0%, 25%, 50%, and 100%, and the effect of the 3rd-stage larvae on the mortality rates of the adult individuals obtained from the larvae was investigated.

2.2. Methods

In the study, 24 *D. melanogaster* eggs were used in the control and experimental groups. In the experimental groups, the samples at the rates of 0%, 25%, 50%, and 100% at 4 different concentrations were added to the habitat of the eggs (Figure 3). The percentage of transformation from eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in media prepared chronically for 15 days were determined. During the experiment, the ambient conditions were maintained at 25 °C and 60% humidity, which are the standard survival conditions of *D. melanogaster*.

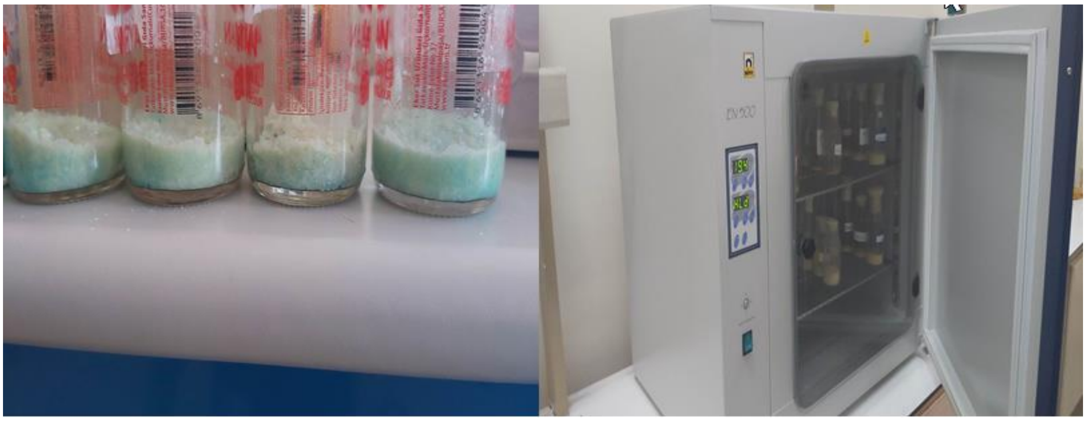

Figure 3. Addition of eggs to the instant *D.melanogaster* medium and standard survival conditions

3. Results

The possible effects of the samples collected from 3 different stations in the Antarctic Peninsula were tested on *D.melanogaster* individuals. Twenty-four eggs were used for each test group. The percentage of transformation of water samples collected from Galindez Island of *D. melanogaster* eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 2.

Table 2. Effects of seawater sample collected from Galindez Island on *D. melanogaster* eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	22	92%
25% P.W*	24	21	20	83.3%
50% P.W	24	12	12	50%
100% P.W	24	9	7	29.1%

*PW, polar water

The percentage of transformation of lake sediment water collected from Ardley Island of *D. melanogaster* eggs to larvae, and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 3.

Table 3. Effects of lake sediment sample collected from Ardley Island on D. melanogaster eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	24	96%
25% P.W*	24	23	23	100%
50% P.W	24	23	22	92%
100% P.W	24	24	23	96%

*PW, polar water

The percentage of transformation of *D. melanogaster* eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 4. In the study, egg-larva-prepupa-pupa and adult stages of all individuals in the control and experimental groups were examined one by one under a stereomicroscope and evaluated (Figure 4).

Table 4. Effects of green algae ice sample collected from Horseshoe Island on *D. melanogaster* eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	22	92%
25% P.W	24	23	23	96%
50% P.W	24	24	22	92%
100% P.W	24	24	23	96%

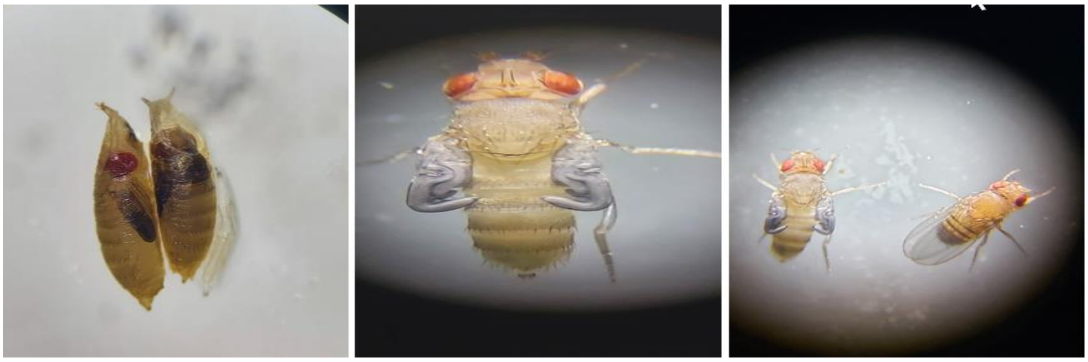

Figure 4. Stages of transformation from eggs to pupae and adult individuals in the control and experimental groups (original)

4. Discussion

In our study, the possible effects of water samples obtained from 3 different regions of the Antarctic Peninsula were evaluated on *Drosophila melanogaster*, an organism used in ecotoxicology studies.

When Table 2 was examined, the effects of the seawater sample collected from Galindez Island were taken into account only at low rates because the water prepared for fruit flies was freshwater. While the survival percentage in eggs and larvae kept alive in the medium containing 25% seawater sample was 83.3%, this percentage decreased due to the increase in seawater. When Table 3 was examined, while the effect of the lake sediment sample collected from Ardley Island on the survival percentage of flies was 96% in the control group, the survival percentages in adult individuals in the experimental groups (25%, 50%, 100%) were found to be 100%, 92%, and 96%, respectively. The fact that the viability rate was determined as 96% in adult individuals kept alive for 15 days in the medium prepared with the water sample obtained from Ardley Island indicated that the water sample from this region did not have any pollution that could have an adverse effect on fruit flies. When Table 4 was examined, the viability rate in the media prepared with water obtained from green algae ice obtained from Horseshoe Island was determined as 96%, 92%, and 96%, respectively (25%, 50%, 100%). The viability rate was determined as 92% in the control group individuals. The same or higher data were obtained with the control group at all rates in the experimental groups. These results showed that the water did not have any toxic effect on the living thing, as in Ardley Island.

When the effect of water samples collected from seawater, green algae ice, and lake sediment samples on the eggs, larvae, and adult individuals of fruit flies was evaluated in general, it was observed that freshwater samples (green algae ice-lake sediment sample) did not cause any negative effects and that the seawater sample caused an increase in mortality rates due to its salinity content.

Although the results we obtained were considered positive for the Antarctic Peninsula, some effects on the increase in the pollution rate in the Antarctic region and the source of pollution in the region were found in the literature review (Light, 2017).

Although Antarctica has been considered an undisturbed region by humans for years, it is also known that the effects of pollutants on Antarctica are increasing every day due to global warming, population growth, and industrial development in countries in the Southern Hemisphere (Kim et al., 2006; Bargagli, 2008). The main human activities in Antarctica are scientific research and the bases established for this purpose, shipping traffic, waste disposal, fishing, and tourism (Aislabie et al., 1999).

Antarctic tourism has expanded with the modern cruise industry that started in 1969 (IAATO, 2020). Large-scale Antarctic tourism increased its popularity at the beginning of the 1990s and also continues to grow nowadays. While the number of tourists was 1000 per year with 12 ships in the 1990-1991 summer season, this ratio increased to 50,000 with a total of 50 ships in the 2017-2018 season (Mc Carthy et al., 2019). According to the latest statistics, the total number of tourists in the 2018-2019 season was 56,168, and the total number of tourists in the 2019-2020 season was expected to reach 78,520 (IAATO, 2020). It is also considered that plastics, which are given to the environment as garbage in proportion to the increasing tourism, pose a threat to the Antarctic environment (Bessa et al., 2019; Lacerda et al., 2019). Therefore, studies are conducted to ensure that all human activities, especially tourism activities, are carried out within the framework of strict rules as far as possible.

Unless the plastic wastes on land are disposed of carefully, they mix with rivers and other water bodies and become a major source of marine pollution (Jambeck et al., 2015). According to global estimates, 80% of plastic waste in the ocean comes from land, while 20% comes directly from the use of plastic in the ocean (Li et al., 2016). Although the first report on marine plastic pollution appeared in the early 1970s, little attention was paid to this problem in the scientific community until the mid-2000s. However, the awareness of marine plastic pollution has increased with the discovery of plastics on the planet's most remote islands (including the Southern Ocean) (Waller et al., 2017).

Plastics in Antarctica can come from a variety of sources. Direct sources such as the disposal of waste from research stations and ships (Waller et al., 2017) and indirect sources such as transport by ocean currents that can transport microplastics from low latitudes to high latitudes of Antarctica can be considered in this context (Fraser et al., 2018). In the study conducted by Eriksson et al. (2013), approximately 6500 samples were collected from six islands in Antarctica (Macquarie Island and Heard Island). It was determined that the lost or discarded fishing gear constituted 22% of the plastics collected on both islands. They were reported to consist mostly of ropes, bait box straps, monofilament ropes, and buoys. In the study conducted by Convey et al. (2002), it was observed that the ocean waste in South Georgia was closely associated with local fishing activities and that the source of plastic waste in the South Sandwich Islands was mainly polystyrene from fishing buoys and remote sources.

Our results showed that no adverse effect occurred in the model organism used, which indicated that the level of pollution in these regions did not reach critical values for the living things used. However, as shown in the examples given above, pollution is occurring in the region and continues to increase due to main human activities, scientific research and bases established for this purpose, ship traffic, waste disposal, fishing and tourism activities.

Although Antarctica is the continent that is the farthest from human impacts, it is adversely affected due to the growing world population and consequently the increasing needs, touristic trips made with the increase of transportation opportunities, and the increasing use of chemicals in industrial applications. Careful implementation of the decisions limiting/prohibiting the use of such chemicals, which have adverse effects by being transported over very long distances, is important in minimizing the ratio of this negative effect.

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