

TOXIC EFFECTS OF ESTER BASED POLYMERS ON *DAPHNIA MAGNA*: A LABORATORY MICROCOSM STUDY

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Abstract: This study aims to determine toxic effects of polyester-based polymers (polycarbonate (PC), polyethylene terephthalate (PET) and, polybutylene terephthalate (PBT)) depending on physiological change in life cycle of *Daphnia magna* Straus (1820) (Cladocera, Crustacea). As a result of acute toxicity test, it was observed that although the rates of immobilized/dead organisms were low, damage started occurring in organisms. According to the results of 72th, the median effective concentration (EC₅₀) values of PC, PET and PBT were determined as 2.604 mg L⁻¹, 4.694 mg L⁻¹ and >100 mg L⁻¹, respectively. In consequence of chronic toxicity test, in the experiment set in which the water where daphnids were cultivated was used, it was observed that there was a high rate of deformation on daphnids which were exposed to ester-based polymers. The possible effect of polyester-based polymers on the daphnids deformation might be consequence of reaction between urea and ester groups of PC, PET, and PBT. Especially in the sets where microalgae existed and the natural conditions were simulated, it was determined that the toxicity response of daphnids varied in accordance with different microplastic types in terms of their chemical structure.

Keywords: Microplastics; ester-based polymer; acute toxicity; chronic toxicity; *Daphnia magna*.

1. INTRODUCTION

Plastics have been used more and more every year because of their remarkable advantages such as mechanical and chemical durability, low cost, easy to process compared too many materials (Barboza et al., 2015; Jemec et al., 2016; Lehtiniemi et al., 2018; O'Connor et al., 2019). On the other hand, microplastics which are defined (the definition varies by researchers) as particles that are smaller than 5 mm or 1 mm (Ivar do Sul & Costa, 2014; Jemec et al., 2016), have great impact on the environment. Microplastic particles can be in different size/shape (microbead, film, fiber, etc.), and they might be obtained from polymers which have different chemical structures and physical properties (Au et al., 2015; Rehse et al., 2016). It was declared that microplastics which have been mostly detected in freshwater ecosystem are polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), polyvinylchloride (PVC), polypropylene (PP) which are the most widely used commercial polymers (Scherer et al., 2018, Cullu et al., 2021). Microplastics can be

released from clothing, packaging, vehicles, electrical and electronic equipments which might be made from polyester-based polymers. Polyesters such as polycarbonate (PC), polyethylene terephthalate (PET) and polybutylene terephthalate (PBT) are engineering thermoplastics and they exhibit high performance in terms of their mechanical and thermal properties as well as chemical resistance. Specifically, the amorphous PC provides high heat resistance, high transparency, good mechanical properties, especially high impact strength and toughness, good resistance toward chemicals such as alcohols, aliphatic hydrocarbons, and dilute acids and also, good weathering properties (DeRudder et al., 2005). PET is the most commercially produced type of polyester which possesses an amorphous or a semicrystalline form which affects the final usage of polymer. PET exhibits high impact, tensile strength, good gas barrier properties and resistance to most chemicals while PBT has a considerably higher crystallization rate and good mold flow properties that make it suitable for injection molding applications in short process cycles. Due to their ease of processing, the

robust and versatile properties of these engineering thermoplastics, they have been used in numerous applications including packaging, textile, electronics, medical devices, and automotive parts (Visakh & Liang, 2015). On the other hand, PC, PET, and PBT are non-biodegradable polymers and by the virtue of the lack of effective solid waste management, they have become major global pollution issue as other polymers.

Plastics, especially colorless, transparent, and micro-fragmented plastics such as packaging film, can become a serious problem by creating “invisible wastes” in water resources. Seawater has already contained various micro- and nanoparticles (per ml $\sim 10^6$ – 10^7 particles) which have sizes more than <100 nm. Swallowing microplastics is the most possible interaction between microplastics and aquatic organisms. It has been known that aquatic organisms especially daphnids exhibit non-selective feeding. The effect of exposed microplastic particles increases because they settle in organs and tissues. Blockage of the gut and disruption of feeding and digestion in animals is one of the most reported adverse effects of microplastics (Jemec et al., 2016). Microplastics are able to reach organisms at different trophic levels by transfer in food webs (Sharifinia et al., 2020). They become vectors as a result of adsorption of monomer structures, plastic additives (such as dispersants, flame retardants, and anti-microbial agents) and different pollutants (persistent organic pollutants and heavy metals) can cause toxic effects on organisms (Guzzetti et al., 2018).

As a basic organism for ecotoxicology, *Daphnia magna* Straus (1820) (Cladocera, Crustacea) (McLaughlin et al., 2005; Olkova, 2021) is not only a major nutrient for fishes but also for the main herbivore of algae, and it forms the main component of freshwater food web (Imhof et al., 2017). The fact that *Daphnia magna* feeds by filtrating the water makes it possible to use this organism to determine the toxicity of microplastics. It cannot make a distinction between planktonic-sized (0.02–200 μm Sieburth et al., 1978) creatures and microorganisms, cannot distinguish or prevent swallowing. Organisms can feed on and hunt on microplastics or can selectively feed on microplastics (Bergmann et al., 2015; Jaikumar et al., 2019). *Daphnia magna* has been found to ingest microplastic fibers at sizes up to 1400 μm in length and 528 μm in width (Jemec et al., 2016) or 106 μm microplastic beads (Frydkjær et al., 2017; Canniff & Hoang, 2018). In the study carried out by Rosenkranz et al. (2009), in the gut of freshwater zooplankton crustacean *Daphnia magna* 20 nm and 1 mm fluorescent carboxylated polystyrene beads, and fluorescent microplastics (polymethyl methacrylate) (29.5 ± 26 nm) were

detected. It is an interesting finding that the microplastic particles found in the digestive system of daphnids are of the same size as sand and plankton (Browne et al., 2008; Jemec et al., 2016).

This study aims to determine toxic effects of polyester-based polymers (PC, PET, and PBT) depending on physiological change in life cycle of *Daphnia magna*. In the literature, aquatic ecotoxicological properties of PET and PC have been evaluated with organisms at different trophic levels (Mansilha et al., 2013; Ogonowski et al., 2016; Ziajahromi et al., 2017; Pignattelli et al., 2021; Setyorini et al., 2021), however this is the first study on the toxicity of PBT on *Daphnia magna*. In this microcosm study, the test medium of the experiment was natural aqueous media where daphnids were cultivated and it contained microalgae. Furthermore, with the aim of determining whether the effects on daphnids were unique to chemical structure of microplastics, toxicity test with PVA, which did not contain ester linkage in its structure and was totally water-soluble, was also carried out.

2. Material and Methods

2.1. Microplastics and their preparation

In the forming/processing processes of plastics, chemical materials such as various reinforcing fibers, plasticizers, colorants, fire retarding materials, and preservatives were added with the aim of improving the physical properties of the process aid and/or the final product. Since there are additives, such as dye etc. in processed polymer structures (plastic) and these may change the result in toxicity test, raw polymer granules, which were not exposed to any process, were used. Therefore, only the toxic effect of the relevant plastic was attempted to be determined.

Commercial grade PC (Lexan™, 141R) was kindly supplied by Sabic. Density, and melt volume flow rate (ISO1133) values of PC were declared as 1.2 g/cm³, 12 cm³/10 min. (300°C and 1.2 kg), by the manufacturer, respectively. Fiber grade, PET (YK-04, semi-dull) and PBT homopolymers (intrinsic viscosity value of 0.645 and 0.83 dl/g at 23°C) were kindly supplied by Korteks, Turkey. Polyvinyl alcohol (PVA 203), of which degree of hydrolyses was 88%, and 4% solution viscosity was 4 cP, was supplied by Sekisui. PET, PBT and PC polymer powders were separated by passing the material through the sieves (Retsch®) with the mesh sizes of <200 , <100 , and <50 μm , respectively. Chemical structures of PC, PET and PBT are given in Figure 1.

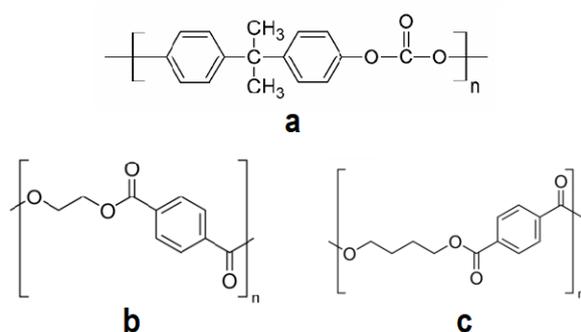


Figure 1. Chemical structure of a) PC, b) PET, c) PBT

2.2. Physico-Chemical Characterization of Microplastics

Polymer samples were analysed using Fourier transform infrared spectroscopy (FTIR) with an attenuated total reflection (ATR) accessory. ATR-FTIR transmittance spectra were measured in the 4000-650 cm^{-1} range using a Perkin Elmer Spectrum 100 FT-IR spectrometer (Spectrum One, USA).

Scanning Electron Microscopy (SEM) imaging of the MP was performed using a Zeiss EVO® LS 10 (Zeiss, Oberkochen, Germany) at 10 kV. For SEM analysis, powder polymers were directly imaged in the electron microscope after the samples were sputter coated with a thin layer of gold.

2.3. Microcosm Study

Microcosm studies were carried out with provided clone of *Daphnia magna*. In order to provide continuity of the clone and to prevent effects caused by the organisms in the test, it was cultivated in IUC MicroLab (Environmental Engineering-Microbiology Laboratory) laboratory conditions. The test medium was obtained from the aquarium by using siphoning method. The produced testing was based *Daphnia magna* Acute and Chronic Immobilisation Test. *Daphnia magna* were fed on *Spirulina* sp. tablet twice a week in 35x70x30 cm aquarium. Ventilation was provided through Atman AT-A8500 (Guangdong, China) air pump motor; temperature was maintained through EHEIM 3612 50W (Deizisau, Germany) brand thermostat control heater; and the darkness and light period was provided through natural sunshine. In order to represent the natural behavior in aquatic ecosystem, the natural aqueous media where *Daphnia magna* lived and stayed was used as the test medium.

The researchers think that the microplastic acute toxicity analysis starts when the microorganisms and microplastics comes together in the laboratory (Harrison et al., 2011; Rogers et al., 2020). However, water, which the organism lives and

continues its natural behaviour, is an integral part of the procedure of analytical process, so analysis starts with selecting water characteristics. This water sampling is so important that, in some cases, it represents the main contribution to the error of the whole process, especially when microplastic contamination is being measured. Even when procedures of acute toxicity analysis are nominally correct, there will be slight variations in the procedures due to ambiguity in measurement protocols and to minor adaptations that are made to protocols in real-world sampling.

An acute toxicity measurement based on physiological or behavioral changes provides a rapid warning in response to a deterioration of the water quality. A number of organisms used and include fish species, daphnia, microorganisms (algae and bacteria) or bivalve mollusks (Mussel monitor). These on-line continuous (real-time) systems provide rapid evaluation and detection of temporal variation in water quality that cannot be achieved through standard approaches to chemical monitoring. But for these types of applications should be used natural waters including water-distribution systems, wastewater effluents, effluents from contamination-remediation sites (where rapid sensing of a change in the water quality is needed). Therefore, in this study, the use of natural in-situ aquarium water, which is a living environment, was preferred, where the organism can maintain its natural behavior without stress.

As the study is a microcosm study, it differs from other toxicity studies. Since laboratory studies carried out on toxicity of microplastics lacked in reflecting natural conditions, results could not be implemented in ecosystems (Lehtiniemi et al., 2018). The test environment used was the natural aqueous media which has the feature of representing the natural aquatic ecosystem in which *Daphnia magna* have been cultivated. It has been known that as the period of exposure increases, the effect of microalgae on the nutrition of *Daphnia magna* decreases. In the study carried out by Canniff & Hoang (2018), it has been demonstrated that microplastics can potentially function as substrate for the growth of algae. In general, it is possible to expand the acute toxicity test up to maximum 96 hours without extra nutrient. As a more realistic scenario, supplementary feed is recommended in case of the possibility that survival rates fall in the control expanding up to 96 hours. However, it is also known that this situation might affect toxicity because of nutrition (Baumann et al., 2014). Also, again according to Baumann et al. (2014), acute toxicity test carried out with *Daphnia magna* cannot be expanded without feeding. It is not possible for newborns to survive without any

nutrition source after 48 hours of the beginning of the test. In the study, since the environment contains microalgae in the experiment sets where natural aqueous media was used as a test medium, it provides the mentioned studying conditions.

2.4. Toxicity Tests

In the test, *Daphnia magna* were exposed to PC, PET and PBT (density 1.20; 1.38; 1.30 g cm⁻³) which has 50 µm and less particle size, which they could consume at different concentrations in acute and chronic ways. To determine whether there would be a difference by the chemical structure of polymer, PVA was studied only as acute toxicity. To prepare microplastics at different concentrations, microplastic particles were measured 3 times with precision scale of RADWAG-AS 60/220.X2 Analytical Balance model (LCGC-Radwag, Poland). *Daphnia magna* (n=10) were placed in each beaker containing natural aqueous media prepared at different concentrations. Different concentrations as "high-dose" and "low-dose" were studied to check the sensitivity and performance of the test organisms. The neonates were put into the test vessels with a Pasteur glass pipette in a small volume, ensuring lowest possible dilution (Baumann et al., 2014). Pre-tests showed daphnids were easily immobilised at different concentrations. As a result of pre-tests, it was studied in 5 different concentrations (5, 10, 20, 50 and 100 mg L⁻¹) with microplastics. Five concentrations of microplastics (PC, PET, PBT), as well as one control group, were used for each experiment and ten daphnids were placed in each control.

Conditions of the test environment were as follows; temperature: 22 ±2 °C, light: darkness 16:8 natural light. It was carried out in exposure for 24, 48, 72 h and lasted 21 days, respectively. It has been known that as the duration of experiment increases, the effect of microalgae on *Daphnia magna* nutrition decreases because of the hunt-hunter relation. In this way, daphnids were forced to get microplastic particles as nutrition.

During acute and chronic exposures, daphnids which were observed to be immobilized were taken from the test environment. At the end of the experiment, immobilized/dead daphnids in each beaker were analysed in KRUSS brand-MBL 2000 binocular microscope (KRUSS, Germany) environment; physical integrity of organisms was analysed and photographed.

To calculate the average effective concentration (EC₅₀) value, immobilized/dead daphnids corresponding to each concentration were used within 95% confidence interval through Probit Analysis. EC₅₀ (mg L⁻¹) results for 1 d (24 h), 2 d (48 h), 3 d (72 h) and 21 days were measured.

2.5. Quality Control and Quality Assurance

IUC MicroLab has with controlled air flow and access and working in laboratories with controlled air flow and minimal personal circulation. In this laboratory was applied air filtration through HEPA filters (Coway, China). All samples were wrapped in aluminum foil and stored in glass containers to prevent air contamination. Protective equipment such as the analysts, a clean cotton lab coat and sterile gloves directly out of the package were used. To prevent contamination caused by stainless steel and glass materials used in the experiment and were sterilized in WiseVen WON Ovens (Champigny-sur-Marne, France).

3. RESULTS AND DISCUSSION

3.1. Characterizations of Microplastics

The structure of PET, PBT and PC were characterized by FTIR, the corresponding results are shown in (Figure 2). FTIR spectroscopy has been one of the crucial characterization techniques to elucidate the molecular structure of polymers. In this study, all polymers contain phenyl and ester groups in the backbones. The aromatic –C–H symmetrical stretching of polymers generates the first peak in the spectrum, which appears at 2969 cm⁻¹. Fingerprint region of carbonyl functional group (C=O stretching vibrations) is found in the range 1750 – 1680 cm⁻¹. The position varies slightly depending on molecular structure of polymers. In PET and PBT samples, this peak is observed at 1710 cm⁻¹, where it is seen at 1750 cm⁻¹ in PC sample. Another common peak refers stretching of the C–O bond appears in two or more bands in the 1300 to 1000 cm⁻¹ range (1222, 1192 and 1163 cm⁻¹ in PC sample, 1454, 1410 and 1340 cm⁻¹ in PET and PBT samples, respectively). PET and PBT samples present absorption bands of terephthalate in the regions at 1240 and 1124 cm⁻¹. Polymers show absorption bands in the 900–700 cm⁻¹, which correspond to the C–H out of plane bending of aromatic rings.

The morphological properties of microplastics affect the toxicity on organisms as well as the chemical structure of plastics (Zocchi & Sommaruga, 2019). Microplastics can easily reach organisms as small as zooplankton as the size gets smaller. Microplastics are evaluated in three classes according to their size. These are lower range microplastics (< 500 µm), upper range small microplastics (0.5–1 mm), and large microplastics (1–5 mm) (Everaert et al., 2018). In this study, effects of lower range microplastics exposure were investigated. Particle size and geometry of powders were investigated by SEM analysis with 1000 magnification. Figure 3 shows that the powders of PC (a), PET (b), and PBT (c). As it can

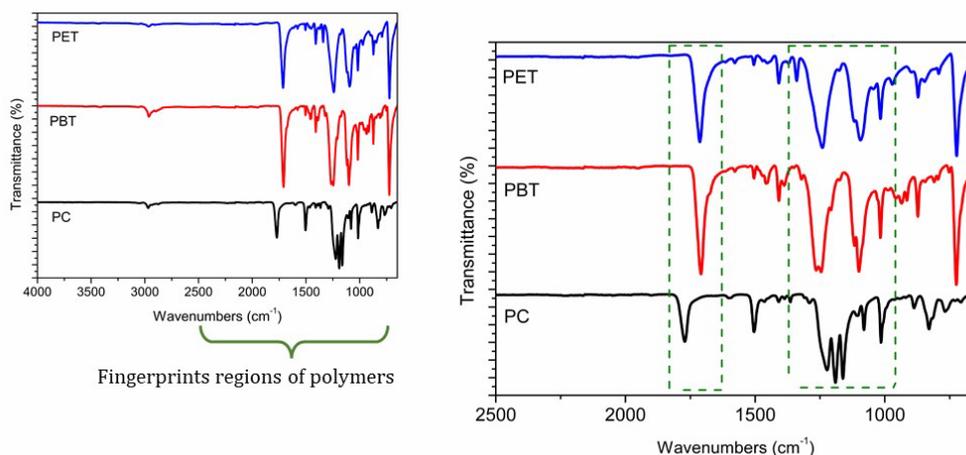


Figure 2. ATR-FTIR spectra of polymers in the 4000–650 cm^{-1} wavenumber region (fingerprint regions marked with a green bands).

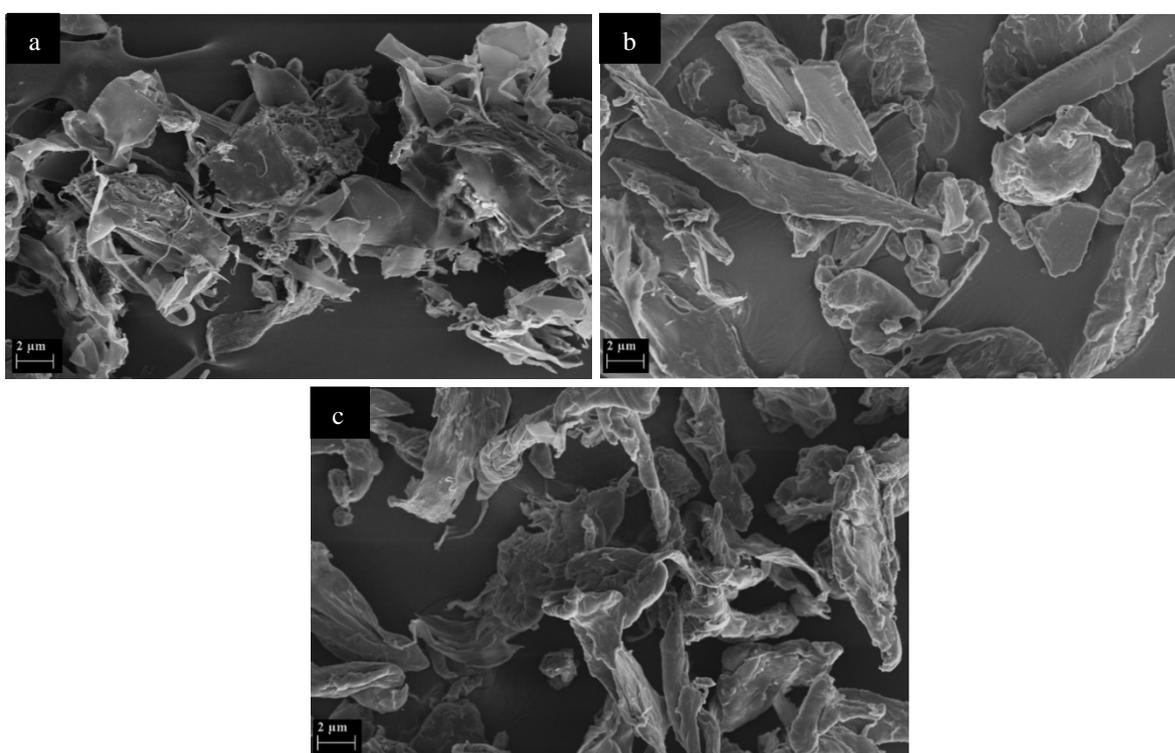


Figure 3. Scanning Electron Microscope (SEM) images of a) PC, b) PET and c) PBT

be seen in figures, mostly irregular shape with the micro- and submicron scale particles were observed. The average dimensions are 500nm – 30 μm , 1 μm – 40 μm and 2 μm – 40 μm for PET, PBT, and PC, respectively.

3.2. Toxic Effects of Different Polymers on *Daphnia magna*

The deformation in organisms by microplastics can be easily compared with *Daphnia magna* in the control sample. Figure 4 shows an image of the living *Daphnia magna* in the control sample.

Six times in each set of experiments were



Figure 4. Microscope image of *Daphnia magna* in control sample (Magnification power 4 \times 10)

studied, and there were response differences in the repetition of the sets as Jemec et al., (2016). Differences in tolerance of *Daphnia magna* seem to be related with the environmental origin of the clone and even after several generations of toxicant-free cultivation, clones originating from ponds surrounded by agriculture, maintain a high tolerance. Not surprisingly, toxicity results obtained even with the “same” test species can be very different, questioning the use among laboratories of different clones of *Daphnia magna* to establish critical parameters such as the EC₅₀ (Zocchi & Sommaruga, 2019).

The rate of immobilized/dead of acute toxicity testing in which PC, PET, PBT polymers are given in Figure 5 and EC₅₀ values of PC, PET, PBT polymers are given in Table 1. 100 mg L⁻¹ is considered high concentration for aquatic ecosystems, at the present time. However, in the next decades environmental microplastic loads are not likely to decrease since the use of plastic increasing day by day. It is estimated microplastic load may be higher than determined at the present (Phuong et al., 2016). When the results of this high concentration (C=100 mg L⁻¹) were examined, almost no damage was observed in organisms which had been exposed to all microplastic types at 24 h. As a result of the calculations made through Probit Analysis, the result could not be calculated because of low rate of immobilized/dead organism for 24th.

Table 1. Results of the acute and chronic toxicity test carried out with PC, PET and PBT

	EC ₅₀ (mg L ⁻¹)			
	24 h (1 d)	48 h (2 d)	72 h (3d)	21 d
PC	*NOB	13.942	2.604	0.371
PET	*NOB	*NOB	4.052	0.090
PBT	*NOB	*NOB	>100	0.051

*NOB: not obtained: The EC₅₀ value could not be calculated because of low rate of immobilized/dead organisms.

At the end of the 48th hour, the immobilized/death rate was determined to be maximum 60% for PC and maximum 20% for PBT (Figure 5). Owing to the high immobilized/death rate, EC₅₀ value for only PC could be calculated (13.92 mg L⁻¹). EC₅₀ values of PET and PBT could not be calculated after 48 hours. In studies conducted on microplastics with different polymer structures (Jemec et al., 2016; Rehse et al., 2016), the EC₅₀ value could not be calculated at the end of the first 24 and 48 hours of exposure. Gerdes et al. (2019), it was determined at the end of the 96th hour that PET (median size ~5 µm) was more toxic than kaolin (~3 µm), a soft clay, as reference material.

For all polymers, the increase immobilized/dead organisms at the end of the 72 h were notable (Figure 5). According to the results,

EC₅₀ values of PC, PET and PBT were determined as 2.604 mg L⁻¹, 4.694 mg L⁻¹ and >100 mg L⁻¹, respectively (Table 1). The reaction of *Daphnia magna* to polymers was PC> PET> PBT.

While the deformation was at its peak level in 100 mg L⁻¹ PC concentration, no deformation was observed in 20 mg L⁻¹ PC concentration and PC concentrations with lower levels (Fig. 6). In the study carried out by Rochman (2015), negative physiological and biological effects were reported in some invertebrates. It was stated that the biological effect was greater on organisms in meso size when the organisms were exposed to microplastics which were smaller than their size. It was also stated by Scherer et al., (2018) that since *Daphnia magna* can easily swallow plastics whose sizes range from nanometer to micrometer; they cause various deformations especially in reproduction organs and various malformations. Adverse impacts have also been documented in various organisms following uptake, such as teratogenicity (Nobre et al., 2015), inflammation (Lu et al., 2016), reduced energy reserves (Wright et al., 2013) as well as reduced feeding (Bergami et al., 2016; Jaikumar et al., 2019). It was detected that in acute and chronic exposure of *Daphnia magna* to polyester staple fibers, it has effects on survival and reproduction (Ziajahromi et al., 2017), and as a result of exposure to polystyrene particles (100 nm), it has effects as varying food uptake rates (Rist et al., 2016). The findings acquired in this study and the results of relevant studies show similarity.

It was observed that the deformation which did not occur in the lower concentrations of the continuing chronic toxicity test (Fig. 7), occurred even in the lowest concentration (5 mg L⁻¹) at the end of the chronic toxicity test (Fig. 8). Similar results were found in the used ester based microplastics. It can be clearly observed in Figure 8 that as the exposure time of *Daphnia magna* to microplastic particles increased, the consumption of microplastic increased; and correspondingly, higher deformation was observed. In the study, it was thought that a chemical reaction was taking place and causing deformation in bodies of daphnids.

According to the findings acquired at the end of chronic toxicity test (at the end of 21 days), PC, PET and PBT of EC₅₀ values was determined as 0.371; 0.090 and 0.551 mg L⁻¹. In the study carried out by Ogonowski et al., (2016), no significant effect on the life history of daphnids exposed to primary microplastic particles was found. Thus, unlike many toxicity studies, both the physiological changes of *Daphnia magna* and the endpoints of the toxicity tests (mortality) were determined. Such difference between acute and chronic toxicity results depends on the increasing number of immobilized/dead organisms as

the exposure time increases. However, it was observed that especially in the chronic toxicity test, the mortality rates due to concentration have inconsistency between themselves. For this reason, one unit increase or decrease in the number of dead organisms in lower concentrations (5 and 10 mg L⁻¹) can change the toxicity class from highly toxic to slightly toxic.

In water flea, PET fibers of up to 1 mm were not

ingested but caused abnormal swimming behavior and carapace and antenna deformities, by interaction from the outside (Ziajahromi et al., 2017) with concentrations within an order of magnitude of reported environmental levels (Ogonowski et al., 2016). In toxicity tests with *Chironomus riparius* (Meigen, 1804) (Thompson & Evenhuis, 1999), it was found that the applied concentration of PET microfibers did not cause adverse

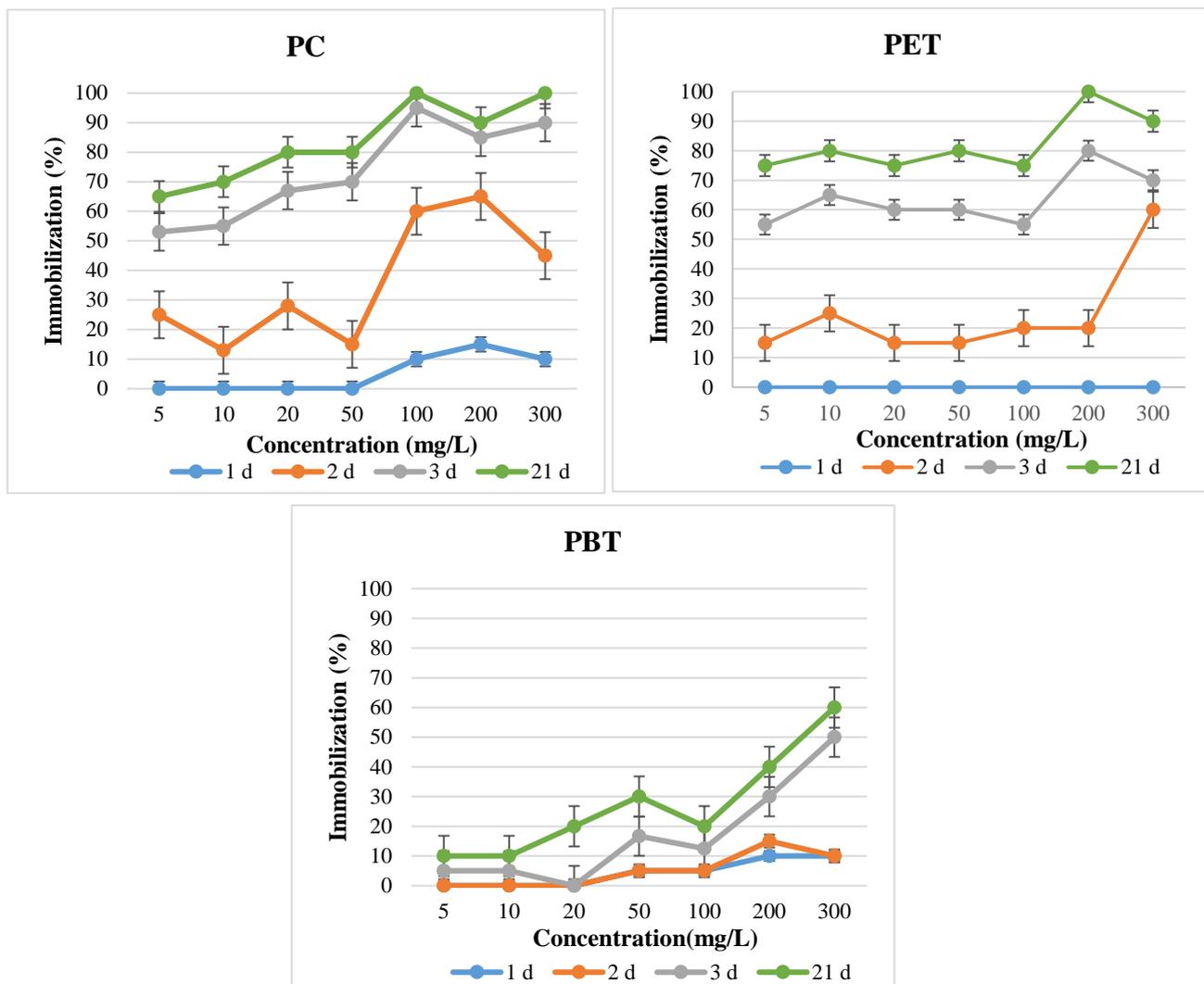


Figure 5. The rates of immobilization of *Daphnia magna* exposed to various concentrations (between 5 and 300 mg L⁻¹) of microplastics (PC, PET, and PBT) related to days (1, 2, 3, and 21 d). Six replicates were run for each test, and error bars show standard deviations.



Figure 6. Microscopic images of dead *Daphnia magna* as a result of acute toxicity test (48 h) of different microplastics at 100 mg L⁻¹ concentration (Magnification power 4×10); a) PC, b) PET, and c) PBT

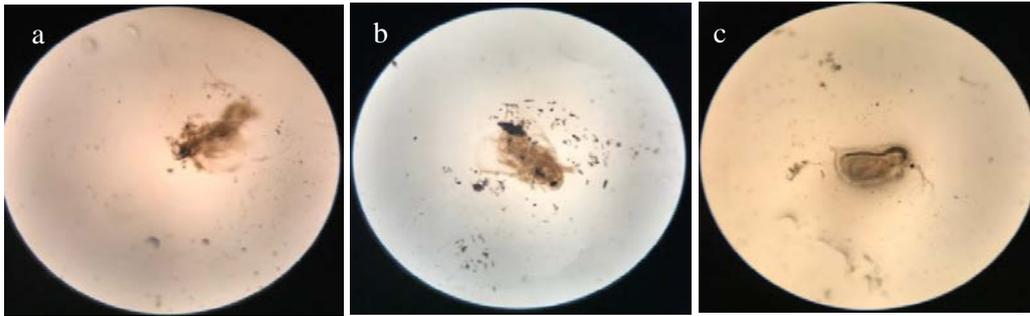


Figure 7. Microscopic images of dead *Daphnia magna* based on concentrations in the continuing chronic toxicity test (14 d) performed in PC (Magnification power 4×10); a) 100 mg L^{-1} , b) 50 mg L^{-1} , and c) 20 mg L^{-1}

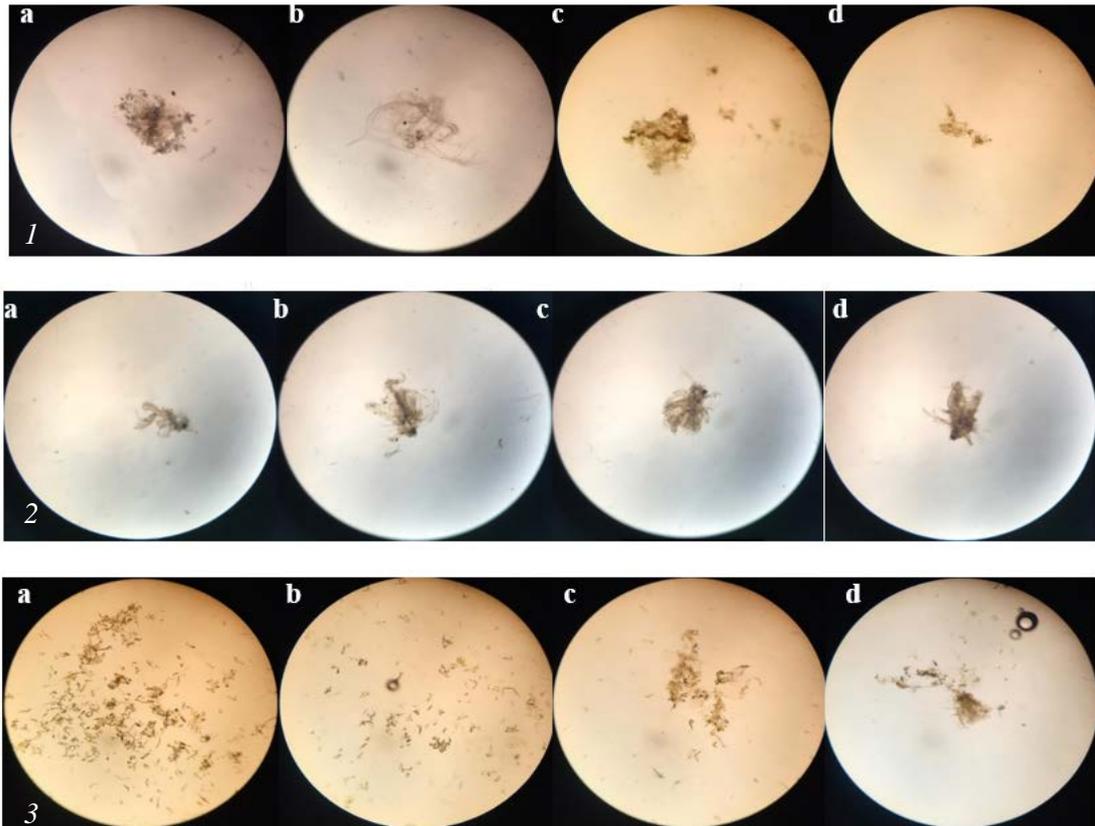


Figure 8. Chronic toxicity test results (Magnification power 4×10) at 21 days; 1 – PET, 2 – PC, 3 – PBT; a) 50 mg L^{-1} b) 20 mg L^{-1} c) 10 mg L^{-1} d) 5 mg L^{-1}

effects at both the organism and the subcellular level in one generation (Setyorini et al., 2021). As a result of the toxicity test with plant *Lepidium sativum* L. (Al-Shehbaz, 1986), it was determined that PET ($60\text{--}3000\text{ }\mu\text{m}$) negatively affected both the biometric and physiological properties of the plant (Pignattelli et al., 2021). PET exposure over 48 d did not affect survival, feeding activity, energy reserves and molting of *Gammarus pulex* (Linnaeus, 1758) (MacNeil et al., 1999). Although bacteria (decomposers) and algae (primary producers) were not affected by acute exposure to PET, larvae at primary consumer trophic levels were found to have increased embryo toxicity under the conditions tested (Piccardo et al., 2020).

Differences may result from variations in the exposure regimes (e.g., duration, particle concentrations), plastic characteristics (e.g., type, size, shape, additives) as well as the species-specific morphological, physiological, and behavioral traits (Weber et al., 2018). Direct exposure of *Daphnia magna* to PC has been found to have significant ecotoxicological effects however, an increase in breeding efficiency was observed as a result (Mansilha et al., 2013).

3.3. Physical Change in the Control Sets

In contrast with these water-insoluble polymers which have ester bonds, PVA which is a

water-soluble polymer with a different chemical structure was used. When the immobilized/dead organisms were examined under the microscope, it was found that the organisms in this experiment set had undergone a different deformation when compared to ester-based microplastic experiment sets (Fig. 9). Figure 9 clearly shows that as the concentration increases, deformation rate increases too. The water-soluble polymer used in the study was chosen not to compare concentration with other microplastics, but to observe the effects of the chemical structure. Therefore, instead of studying parallel concentrations, concentrations which had been caused deformations in polyesters were studied. It was aimed to prove that the deformation observed in this structure had been different from the deformation seen in other microplastics studied. In this context, it would not be possible to observe the same deformation for each polymer structure. Deformation in daphnids exposed to PVA might also be due to density difference. However, the reason for deformations of the studied microplastics in daphnids was not a density difference. Similar deformation/death was observed as the chemical structures of these microplastics were the same. It was also expressed by Rehse et al., (2016) that when microplastic particles were distributed to the water column in sizes that could be taken by *Daphnia magna*, both dose and time-related effects could be seen. In a further study conducted by Besseling et al., (2014), some findings were acquired showing that there are serious changes in reproduction and downsizing in the body of *Daphnia* sp. which was exposed to nano-sized polystyrene particles. In this study conducted with PVA, it is noteworthy that there is growth despite the decrease in body size observed in the study of Besseling et al., (2014).

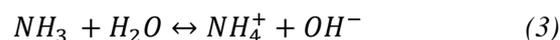
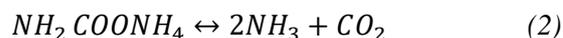
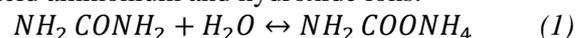
3.4. Effect of Feeding on *Daphnia magna*

In the presence of microalgae, microplastic

uptake of daphnia was not involved. Therefore, no direct feed was made at regular intervals. The microalgae in the aquarium water used as the test medium were provided to be consumed over time. Due to the gradually decreasing microalgae abundance, it has been considered that the cause of death of daphnids in chronic toxicity was not lack of nutrition. Furthermore, at the end of the acute toxicity test (after 48 hours), there was no possibility of fasting daphnids, especially in the test medium (in the presence of microalgae) with aquarium water. In order to comprehend the cause of the deformation observed in daphnids, control groups were also examined under a microscope. The deformation of the daphnids exposed to the studied microplastics was not found in the control group. In addition, findings similar to the deformation observed at the end of the chronic toxicity test were encountered at the end of the acute toxicity test and the ongoing chronic toxicity test.

3.5. The Effects of Chemical Structure of Polymers on the Deformation of *Daphnia magna*

Wiltshire & Lampert (1999) had evaluated that 0.06-0.1 $\mu\text{g mg}^{-1} \text{h}^{-1}$ of urea, 0.31-1.2 $\mu\text{g mg}^{-1} \text{h}^{-1}$ of ammonia were excreted by *Daphnia magna*. The excretion amounts depended on feeding animals (Wiltshire & Lampert, 1999). It is well known that urea decomposes into ammonia as follows. Ammonia slightly dissociates in water at room temperature to yield ammonium and hydroxide ions.



Several studies have reported that urea and its derivatives in aqueous media have noticeable influence on the ester linkages of polyester-based polymers and contribute to decomposition of polymers (Wang et al., 2015; Nomai et al., 2017; Zumelzu et al., 2017).



Figure 9. Microscopic images of organisms detected as a result of acute toxicity test (48 h) in different concentrations of PVA (Magnification power 4×10); a) 50 g L⁻¹ b) 20 g L⁻¹ c) 10 g L⁻¹ and d) 5 g L⁻¹

Although, the urea content is very low in the Daphnids tank, urea may catalyze hydrolysis of esters to form ketene intermediates which easily react with water to form acetic acid, that reaction is also accelerated by the presence of alkalis (Cameron et al., 1937). Acute toxicity of ketene on human beings has not been studied yet. On the other hand, it was studied that inhalation exposure of ketene has similar clinical effects with phosgene. The toxicologic profile of ketene is similar with phosgene in various animal species such as rats, cats, and rabbits (National Research Council, 2014).

Another possible reaction is that urea may react oxygen of (carbonyl groups) polyester-based polymers to form ammonium carbonate or ammonia; those can be taken up by aquatic organisms through respiration and accumulate in their structure and as a result of this exposure, Daphnids deformed. (CAS Reg. No. 1111-78-0; Osada et al., 2011).

4. CONCLUSIONS

The relationship between the ecological consequences which may occur because of exposure of aquatic organisms to microplastics, and the chemical structure of these contaminants were attempted to be revealed. As a result, it was observed that different deformations occurred on organisms in acute and chronic exposures because of the main factors affecting the toxicity, such as exposure time and concentration. When acute exposure was analysed, it was found out that deformation was observed in the sets compared to the control organism. In the chronic exposure, which is generally considered more representative for ecotoxicological risk assessments compared to acute exposure, high level deformation was detected in all sets using natural aqueous media as a test medium. It was observed that deformation ways of organisms which were exposed to ester based microplastics (PC, PET, and PBT) were different from the organisms which were exposed to PVA.

The toxicity of microplastics is attempted to be explained by the adsorption on microplastic particles of the intermediate organic compounds which are formed as a result of partial decomposition of the polymers and the additives used in the processing of the polymer forming the basic structure of plastics. This study is an innovative study that predicts that each microplastic, which has not included any additional processing aids, may cause different effects on organisms due to different chemical structures. The possible effect of polyester-based polymers on the daphnids deformation might be consequence of reaction between urea and ester groups of PC, PET, and PBT. With this study, the

biological and chemical effects of the toxicity determined in the closest conditions to the field studies have been revealed from the perspective of quantity, and it has provided a different perspective to the subject. Through this study, it was clearly observed that chemical structures of polymers influence deformation (dead or explosion as in ours) types of *Daphnia* sp.

Among the polymers used in the study, PC, PET and PBT have different chemical structures compared to PVA; and its toxic effect on organisms varies. In order to identify the risks arising from microplastic contamination in ecosystems in a better way, it is important to clearly demonstrate the effect of different microplastic types on organisms. According to this, further research is needed to determine the single and multiple toxicities of different polymer types as virgin or processed plastics. It is particularly important to examine the toxicity of other polymers other than PS and PE.

Compliance with ethical standards Ethical Approval

The research meets all applicable standards regarding the ethics of experimentation and research integrity, and the following is being certified/declared true. Our study does not involve human subjects. As an expert scientist and along with co-authors of concerned fields, the paper has been submitted with full responsibility, following due ethical procedure, and there is no duplicate publication, fraud, plagiarism, or concerns about animal or human experimentation.

A Disclosure / Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. It is to specifically state that "No Competing interests are at stake and there is No Conflict of Interest" with other people or organizations that could inappropriately influence or bias the content of the paper.

Availability of data and materials

Not applicable.

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Authors Contributions

V.Z.S. and N.S. conceived the presented idea. V.Z.S. conducted the literature review and data collection. V.Z.S. verified the analytical and lab methods. V.Z.S. investigated and supervised the findings of lab works. N.E. supervised the findings of chemical structure and ATR-FTIR Analysis. N.S and N.E. edited and reviewed all

manuscript drafts. All authors discussed the results and contributed to the final manuscript.

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