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Evaluation of Microplastic Toxicity in Drinking Water Using Different Test Systems

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Abstract: Microplastic pollution poses a significant threat to environmental and human health. This study investigated the toxicological and genotoxic effects of various microplastic types (polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE)) on plant and animal models. Aqueous extracts of microplastics in different size fractions (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm) were evaluated for their impact on barley seed germination and cell division. Results indicated that smaller microplastic fractions exhibited higher toxicity, particularly for PP and PE. Significant reductions in germination rates and root growth were observed, along with increased chromosomal aberrations in barley cells. Furthermore, the migration of formaldehyde, a known toxicant, from microplastics exceeded permissible limits. These findings highlight the potential risks associated with microplastic pollution, particularly in drinking water sources. Future research should focus on the long-term health impacts of microplastic exposure, including carcinogenic potential, and explore the synergistic effects with other pollutants. Stricter regulations on microplastic pollution and advancements in water treatment technologies are urgently needed to mitigate these risks.

Keywords: microplastic; phytotoxicity; mutagenicity; toxicant migration; acute toxicity

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1. Introduction

The widespread use of polymeric materials has introduced a novel source of environmental pollution. Although polymeric materials are inherently inert, they can release unreacted and unincorporated toxic oligomers and monomers into the environment, thereby imparting toxic properties [1,2]. Additives used to enhance the properties of polymer products (such as plasticizers, modifiers, solvents, and other raw material components) are also toxic and, in most cases, chemically unrelated to the plastic polymer. In such instances, non-covalently bound chemical components may migrate from the polymer product into the surrounding media [3,4]. While the migration of chemicals from plastics typically occurs in small amounts, it can persist over extended periods [5]. The potential hazard posed by microplastics to living organisms is exacerbated by their high sorption capacity for environmental toxicants, attributed to their high surface area-to-volume ratio [6]. Consequently, the risk of contamination of the soil, air, and water by toxic migration products from polymer matrices may be significant.

The situation is exacerbated by the widespread distribution of degraded plastic particles (microplastics) in various environmental compartments, including surface waters, soils, and atmospheric air [7–9]. A key source of microplastic introduction into the natural

environment is landfills, where waste plastics, primarily under the influence of abiotic factors, degrade into smaller fractions and subsequently migrate into the environment [10].

The literature indicates that microplastics can exert physical, chemical, and biological effects on living organisms and their habitats. Previous studies demonstrated that microplastics can influence plants by altering the physical structure of the soil, thereby affecting the availability of water and nutrients [11]. Several researchers have reported that microplastics are capable of adsorbing and transporting toxic substances, such as heavy metals and organic pollutants, thereby amplifying their toxic effects on plants. Additionally, microplastics may contain or release substances that disrupt physiological processes in plants [12–16].

Published studies highlight the dangers of microplastics to aquatic organisms and humans [17,18]. One of the primary mechanisms of exposure to microplastics is their ingestion through the gastrointestinal tract of living organisms via contaminated food and water, ultimately making their way up the food chain into the human body. Microplastics have been detected in various foods, including non-alcoholic beverages, fish, vegetables, fruits, packaged foods, and honey [19–21], as well as in drinking water (both bottled and tap water) [22,23]. Additionally, scientists have provided approximate estimates of microplastic intake in humans, based on their presence in different food and water sources [24].

The indirect negative impacts of microplastics are associated with their ability to transport sorbed chemicals. Microplastics can contain a wide range of chemical additives, such as bisphenol A (BPA), phthalates, and polybrominated diphenyl ethers, which are used in plastic synthesis to enhance plasticity [25,26]. These additives can disrupt the endocrine system and exert toxic effects on the human body when they migrate from the polymer matrix [27]. Due to their small size, microplastics can easily be ingested by aquatic organisms, enter the bloodstream, and accumulate in the gastrointestinal tract and other organs, thereby transporting chemicals and inducing oxidative stress [28–30].

The available data on microplastics in food and drinking water are difficult to generalize due to the absence of standardized sampling and analysis protocols, as well as inconsistencies in the qualitative and quantitative expression of results. The assessment of microplastic toxicity is even more challenging; studies in this field are not only limited but also vary in terms of plastic types, particle size, exposure doses, and test models. Nevertheless, the outcomes of these studies consistently demonstrate various manifestations of microplastic toxicity. For example, ref. [31] investigated the effects of single-dose oral exposure to polyethylene terephthalate (PET) microplastics in adult male Wistar rats, revealing tissue and organ damage and dysfunction. Similarly, hepatotoxicity was observed following oral and nasal exposure to nano/microplastics of polylactic acid in experiments on mice [24].

These findings underscore the urgent need for further research into the toxicity of microplastics, particularly those in contact with food and water. Such research will support the development of effective strategies for managing the production and use of plastic products in everyday life, thereby mitigating the exposure pathways of toxic plastics to the human body.

Although the number of experiments studying the toxicity of microplastics is constantly growing, there are still insufficient data to assess the toxic effects on human health, including the lack of epidemiological studies. Most studies conducted on animals and human cells indicate the presence of oxidative stress, failure of the antioxidant defense system against toxicity caused by microplastics, and induction of reproductive and developmental toxicity [32,33]. In separate experiments, genomic instability was observed at low concentrations of PE microplastics, as revealed in an experiment on human blood lymphocytes [34], as well as a negative impact of PS particles on immature cardiomyocytes (in an experiment on newborn rats) [35], functional disorder of respiratory tract organelles (on polyester particles) [36], and the disruption of the function of forebrain, liver, and intestinal organelles, established on polystyrene particles [37]. Moreover, the toxic effects, such as decreased cell viability, depended on the dose of exposure [38].

Although microplastics are too large to penetrate the skin, they can cause skin irritancy when in contact with creams, soil, children's toys, etc. [39,40].

Additives in plastics are thought to be toxic, carcinogenic, and mutagenic, but due to the impossibility of conducting human studies, the exact effects on the body have not yet been studied [32].

A review of 133 articles [33] describes the presence of adverse effects of nano- and microplastics on human health, but 78.9% of the studies are devoted to the areas of PS and 12% of the studies describe conflicting results. The presence of studies indicating the absence of a toxic effect (under certain experimental conditions) [41] supports our proposal for the need for additional toxicological studies under harmonized/standardized experimental conditions. It is therefore hoped that this study, which covers four types of microplastics of different sizes, the toxicity of which was studied simultaneously using different methods, expands the existing knowledge about the potential toxicity of microplastics.

Determining the mechanisms of the toxic effects of microplastics on living organisms is an important task, but at the same time is not fully understood. At present, it has been established that microplastics can cause genetic damage through mechanical damage to cells, chemical pollution, and interference with cellular processes [42]. Microplastics can block the pores of seeds, including due to the presence of plasticizers [43], and thus prevent water absorption [44]. In turn, a decrease in the swelling process leads to a decrease in the rate of germination [45]. Microplastics can penetrate the root system of plants, disrupting their normal functions [46]. The toxic effect of microplastics may also be linked to their ability to cause DNA breaks in cell cultures [47,48] and influence dominant pathways leading to the modulation of inflammation and cell proliferation [38]. The ability of microplastics to carry toxic chemicals such as heavy metals and organic pollutants on their surface exacerbates their toxic impact [49,50].

Our previous studies on microplastic monitoring have confirmed its presence in all surveyed environmental samples, including natural waters and sediments, in the Akmola region of Kazakhstan [51,52]. The existing water supply system in Kokshetau, as in Kazakhstan as a whole, is based on the predominant use of surface water as drinking water (96.1%), while 94.7% of the population is provided with a centralized water supply [52]. The critical condition of water supply systems (built in the 1960s–1970s) and outdated water treatment technologies (coarse filtration, filtration through sand filters, coagulation, and flotation, settling, and disinfection) do not ensure effective water purification from insoluble microimpurities [53]. In addition, the failure to comply with water treatment regimes in the city of Kokshetau, the absence of coagulation and flotation workshops, and the small number of sand filters and their low productivity lead to the presence of microplastics in samples of tap water in the city of Kokshetau with a size of more than 300 μm in a concentration of (2.0×10^{-2}–6.0×10^{-2}) particles/dm^3 [52]. The weak regional environmental policies regarding plastic waste management lead to the widespread presence of plastic waste in unauthorized landfills on the coast of water bodies, which ultimately leads to the entry of microplastics into natural and drinking waters [54]. Assuming a systemic intake of microplastics into the body of the population of Kokshetau with drinking water, the toxic properties of individual types of microplastics, mostly found in tap water in Kokshetau, were studied [52]. Investigating the toxicity of the types of microplastics found in the tap water of Kokshetau city, Akmola region (Kazakhstan) will facilitate predictions of the toxic risks associated with drinking water.

Focusing on the assessment of toxicity not from the polymers themselves, but from their constituent components that migrate, our study evaluated the toxicity of aqueous extracts from polymer particles fragmented to microplastic size. Similarly, the Office of Food Additive Safety (OFAS) of the U.S. FDA's Center for Food Safety and Applied Nutrition assesses food-contact substances by examining the toxicity of migrating toxicants rather than the polymers themselves [55]. In the context of polymers, the OFAS investigates oligomers and low molecular weight compounds capable of migrating into the contact medium, as they are considered more biologically relevant. We propose that studying the

toxicity of polymer material extracts serves as a reliable indicator of a plastic's potential to impart toxic properties to various liquids. A negative result in such studies may indicate the harmlessness of the polymeric material under the specific experimental conditions (such as exposure temperature, microplastic type, and particle size).

The investigation of microplastic aqueous extract toxicity brings us closer to understanding the safety of exposure to drinking water in contact with plastics, such as when using plastic water pipes or plastic water storage tanks.

Recognizing the insufficiency and ambiguity of assessing the toxic properties of synthetic materials through a single method, along with ethical considerations such as avoiding human experiments and reducing animal testing, the aim of this study was to comprehensively assess the toxicity of aqueous extracts from microplastics (PP, PE, PET, and PS) with different degrees of dispersibility. This assessment was conducted using plant and animal test organisms, frozen bull semen, and through an analysis of the migration of organic substances into the aquatic environment.

The use of plant test objects (phytotoxicity assessment) provides a method to evaluate toxicity without resorting to animal or human testing, and it also helps to assess the potential impact of microplastic extracts on phytocenoses. Additionally, some studies suggest that animal tests may not always exhibit high sensitivity to certain toxicants [56]. Since seed germination has a significant impact on plant yield, it is one of the most common indicators for assessing the phytotoxicity of environmental pollutants [57,58].

Studies on the genotoxicity of microplastics are very limited, yet this assessment is crucial, particularly in relation to drinking water that comes into contact with plastic products. Toxic substances released into the environment disrupt natural cellular processes, leading to structural modifications in DNA and chromosomal abnormalities [12,59–61]. We consider the detection of chromosomal damage in plant test objects to be an effective method for studying the genotoxic activity of microplastics [62,63].

Animal studies (using rabbits in this study) were deemed necessary to investigate the epicutaneous effects of water extracts from the studied microplastics. It is hypothesized that toxic substances migrating from microplastics into water may have an irritant effect on human skin during activities such as bathing. The use of rabbit skin in studies of skin irritancy in rabbits has long been known, as they show good correlations between the results obtained in rabbits and the results obtained in humans, including for non-irritating or highly irritating substances [64,65]. Furthermore, the skin of rats and hamsters is not sensitive enough to be useful in studies of the skin-irritating properties of toxicants [65]. The in vivo rabbit skin irritation test is currently the primary method for testing skin irritation and is the reference method against which non-animal alternatives are compared [66].

One effective method for assessing the acute toxicity of chemical substances is the evaluation of changes in sperm motility using bovine semen. Many researchers consider this method to be more sensitive than tests for hemolytic or toxic effects of plastic products [67]. The method is simple to perform, can be used for large-scale studies, and has high reproducibility. These advantages make it suitable for use in the Kazakhstan system for certifying plastic products, as well as for other Eurasian Economic Union (EurAsEU) countries [68–70]. Although studies on the toxicity of substances often involve cattle semen, they are typically conducted on metal particles (e.g., magnetite [71], gold [72], or iron [73]). Research on the toxic effects of microplastics on semen parameters and reproductive function is scarce. However, one of the few studies on polystyrene microparticles demonstrated decreased sperm functionality and increased oxidative stress in embryos [73]. Our study will contribute to the investigation of fertility and male infertility, which has gained relevance given the widespread accumulation of microplastics in the environment [74,75].

The migration behavior of microplastic components is also poorly studied, likely due to a lack of awareness of the potential public health risks posed by toxicants migrating from polymeric materials. Phenol and formaldehyde are two common chemicals known to migrate into contact media from polymer products [76]. Formaldehyde can cause degenerative changes in parenchymatous organs and skin sensitization, and has significant effects

on the central nervous system. It also inactivates several enzymes in organs and tissues, inhibits nucleic acid synthesis, disrupts vitamin C metabolism, and exhibits mutagenic properties. Formaldehyde is particularly hazardous due to its ability to be rapidly and completely absorbed through any route of entry into the body [77].

The comprehensive nature of this study will provide a more thorough evaluation of the potential hazards posed by aqueous extracts from microplastics of various structures and sizes to living organisms. The findings can be used to assess the potential risks to humans and biota in contact with water containing microplastics.

2. Materials and Methods

2.1. Objects of the Study

All toxicological studies on microplastics were conducted using four types of plastics: polypropylene (PP, from water pipes), polyethylene (PE, from water pipes), polyethylene terephthalate (PET, from plastic bottles), and polystyrene (PS, from packaging containers). These types of plastics were selected based on their prevalence in natural and drinking waters, as established in our earlier studies [51,52]. The structures of the selected polymers were determined by infrared (IR) spectroscopy using an IR-Prestige 21 spectrometer (Shimadzu Corporation, Kyoto, Japan) within the wavelength range of 4000–400 cm^{-1}. No special sample preparation was required, and the analysis was conducted using a DuraSampl IR II single-reflection total internal reflection (ATR) attachment (prism material: diamond on ZnSe substrate) (Smiths Detection, Danbury, CT, USA).

The IR spectra were analyzed using the Polymer2, Polymer, T-Polymer, and T-Organic library databases, as well as by interpreting absorption bands corresponding to the stretching and bending vibrations of functional groups characteristic of specific polymers. Polypropylene (PP) was identified by the presence of absorption bands corresponding to the stretching and bending vibrations of CH, CH_2, and CH_3 groups at 2950, 2918, 2836, 1456, and 1376 cm^{-1}. Similarly, the IR spectrum of polyethylene (PE) showed absorption bands at 2916, 2846, 1468, and 717 cm^{-1}, corresponding to the stretching and bending vibrations of the CH_2 group [52,78]. The structures of polyethylene terephthalate (PET) and polystyrene (PS) were identified by comparing their Fourier-transform infrared (FTIR) spectra with those in polymer library databases.

All types of plastics were manually shredded and further processed through various laboratory mills to simulate the fragmentation of microplastics as they naturally degrade in the environment. In order to standardize all studies throughout the project, we studied the fractions of microplastics obtained by us with the available set of sieves (0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm). Thus, in the toxicological study, we covered the range of sizes of microplastics found by us in surface waters and in tap water (100–500 µm on the largest side [51,52]), which correlates with other published data, where the detected microplastic particles were in the range of more than 0.7 mm, less than 0.3 mm, and in the range of 0.3–0.7 mm [79]. Given that the entire surface area of a plastic container or a significant surface area of a plastic water pipe may be in contact with water, in this study we also included microplastics larger than 1 mm (1 mm and 3 mm), taking into account that the maximum microplastic size is generally considered to be particles smaller than 5 mm [80].

Thus, the shredded plastic was then sieved into fractions of 3 mm, 2 mm, 1 mm, 0.3 mm, and 0.175 mm using a series of sieves. All experiments were carried out on the following 20 different variants of microplastic:

- Aqueous extracts from plastic bottle (PET) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;
- Aqueous extracts from plastic container (PC) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;
- Water extracts from water pipe (PP) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;

- Water extracts from water pipe (PE) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm.

In order to simulate the contact of drinking water with the surface of plastic containers and to ensure the prevention of external contamination, the process of microplastic extraction was carried out in closed containers. After the specified extraction time, the microplastic was separated from the liquid fraction by filtration through No. 42 Whatman filters, which ensured the removal of any particles larger than 2.5 μm. The resulting extracts were then used to assess their toxic properties. The toxicity of aqueous extracts of microplastics was evaluated in a toxicity study:

- Phytotoxicity;
- Genotoxic properties on a plant test subject;
- Local cutaneous irritant action at single applications to the back skin of experimental animals;
- Phenol and formaldehyde migration;
- Acute toxicity using frozen bovine semen.

The test subject in the experiment to assess phytotoxicity and mutagenic activity was common barley (*Hordeum vulgare* L.), often used by scientists as a model species for basic and applied research on agricultural crops. The diploid genome and haploid set of a small number (7) of chromosomes makes barley suitable for genotoxic studies [81,82]. In addition, barley is the most common type of agricultural crop grown in the Akmola region and in Kazakhstan. Given the fact that microplastic pollution has the greatest impact on agrocenoses [83], this study on the toxicity of microplastics on barley is informative for such agrocenoses.

The positive control was methyl methanesulfonate (MMS), a classic mutagen [81,82], and distilled water (dH_2O) was the negative.

Sexually mature laboratory animals, specifically rabbits weighing between 3500–3800 g, were selected as test subjects for studying the epicutaneous effects of aqueous extracts of microplastics. The selection of animals and the formation of homogeneous experimental and control groups were carried out with consideration of similar body weight (with a maximum weight difference of no more than 10% within each group), as well as the absence of differences in behavior and general health condition.

For the acute toxicity experiment, bovine semen frozen in liquid nitrogen vapor was used as a test subject. The frozen bovine semen pellets were obtained from artificial insemination stations and stored in Dewar vessels filled with liquid nitrogen.

2.2. Research Methods

To assess the phytotoxicity and genotoxicity of aqueous extracts of microplastics, all types of shredded plastic were divided into fractions and immersed in distilled water at a concentration of 1 g of microplastic per 100 cm^3 of water. The mixtures were then incubated for 1 month at thermostatic room temperature (22–25 °C). For experiments evaluating skin irritant effects, the migration of phenol and formaldehyde, and acute toxicity using frozen bovine semen, the preparation of aqueous extracts of microplastics was conducted according to the relevant standards described in Sections 2.2.1–2.2.5

In order to standardize the experimental conditions, aqueous extracts were obtained using distilled water with a pH of 5.4–6.6 (without further changing the acid–base balance), at illumination levels regulated by standards for laboratory and thermostatic rooms (400 lux) and for the vivarium (325 lux) [84], and the following thermostatting temperature conditions:

- Assessment of phytotoxicity and frozen bovine semen: obtaining extracts—22–25 °C, seed germination—23–24 °C;
- Assessment of skin irritant properties: preparation of aqueous extracts—(18–24 °C);
- Acute toxicity studies on bovine semen—preparation of aqueous extracts at 40 °C;

- study of phenol and formaldehyde migration—preparation of extracts at a temperature of −22–25 °C.

2.2.1. Method for Analyzing Phytotoxic Properties

To conduct research on the model test subject barley (*Hordeum vulgare* L.), seeds with a germination efficiency of at least 80% were selected at the initial stage. The scheme for determining phytotoxicity is shown in Figure 1a.

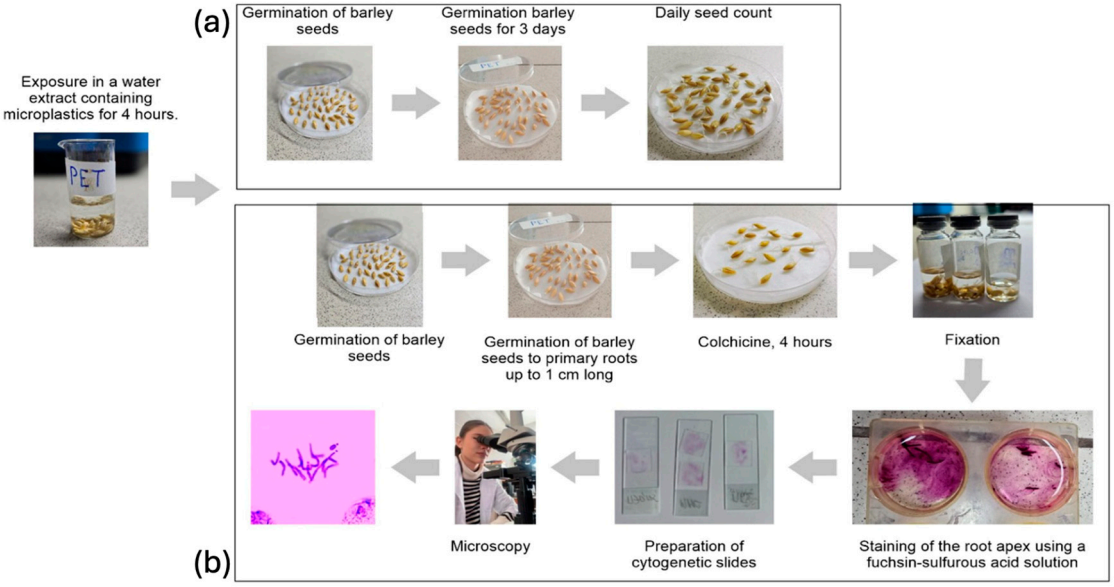

Figure 1. Schematic diagram of (**a**) analysis of phytotoxicity and (**b**) mutagenic activity of microplastic extracts.

The cleaned seeds were soaked in water for 12 h to enhance germination. Following this, the already swollen seeds (50 per treatment) were immersed in aqueous extracts of microplastics for 4 h. After soaking, the seeds were planted in Petri dishes and placed in a thermostat at 23–24 °C for root germination over a period of 3 days. The number of germinated seeds was recorded every 24 h. Seed germination was determined by the presence of a visible root emerging from the split seed coat [44].

The phytotoxic activity of the various microplastic treatments was assessed based on the germination rate, seedling vigor, and overall germination rate of common barley seeds (*Hordeum vulgare* L.) as per the methodology outlined in [85]. The percentage of seed germination (B) was calculated using Equation (1).

$$B = \frac{a}{b} \times 100\% \quad (1)$$

where a is the number of germinated seeds and b is the total number of seeds.

The germination rate (C) is the sum of the average number of seeds germinating daily, calculated using Equation (2).

$$C = a + \frac{b}{2} + \frac{c}{3} \quad (2)$$

where a is the number of seeds germinated during the first day, b is the number of seeds germinated on the second day, and c is the number of seeds germinated on the third day.

Germination unity refers to the average percentage of seeds per day of germination, calculated by Equation (3):

$$D = \frac{P}{A} \quad (3)$$

where D is the germination unity, P the number of germinated seeds for the first day, and A the number of germinated seeds for the fourth day.

2.2.2. Method of Mutagenic Activity Analysis

The general scheme of the analysis of mutagenic activity is presented in Figure 1b.

Methods of Preparation of Cytogenetic Preparations from Cells of the Root Meristem of Common Barley (*Hordeum vulgare* L.)

Cytogenetic preparations from the cells of the root meristem of common barley were prepared and stained with fuchsin–sulfuric acid according to the methodology outlined in [85]. To arrest cell division at the metaphase stage, seeds germinated overnight in the thermostat were transferred to a 0.01% colchicine solution for 4 h. The seeds were then fixed using a freshly prepared solution of glacial acetic acid and 96% ethyl alcohol in a 1:1 ratio.

Initially, the fixed material was thoroughly washed in three portions of distilled water, with each wash lasting 4 min. Following the water washes, cold hydrolysis was performed using a pre-cooled solution of concentrated hydrochloric acid diluted with water at a 1:1 ratio for 50 min at 4–5 °C. After hydrolysis, the samples were washed to remove hydrochloric acid and incubated in a fuchsinic acid solution for a minimum of 12 h in the dark.

The enzymatic maceration method was employed to decompose the intercellular matrix and remove the cell walls. During this stage, the brightly colored root tips containing the zone of actively dividing meristematic cells were carefully excised with a scalpel and placed in an aqueous solution of cytase for 30 min at 30 °C. After washing the enzyme off with distilled water, 2–3 drops of 45% acetic acid were added to the apical parts of the roots and allowed to incubate for 1–2 min. The root tips were then transferred to a microscope slide using a pipette and covered with a coverslip to create a monolayer of cells.

Microscopy was the final step, in which the cytogenetic preparations were analyzed under an MX 100T microscope (MicroOptix, Wiener Neudorf, Austria) at a magnification of 1000×.

Metaphase Method of Accounting for Chromosomal Aberrations

The genotoxic activity of microplastic aqueous extracts was assessed by counting metaphase chromosomal aberrations. For each experimental variant, more than 400 metaphase cells from barley root meristems were examined. In analyzing the structural changes of chromosomes, both the overall frequency of chromosomal aberrations and the frequency of chromosomal and chromatid-type abnormalities were considered. The frequency of aberrations resulting from natural mutation processes in plant cells served as the control. For each experimental variant, the percentage of cells with altered chromosomes was calculated relative to the total number of metaphases studied, as well as the percentage of total chromosomal changes classified as chromosome rearrangements.

Experiments assessing phytotoxicity and genotoxicity were conducted in triplicate. For all cases, mean values and their associated standard errors were computed using the Microsoft Excel data analysis package. The significance of differences between mean values was evaluated using Student's *t*-test for independent samples, with a significance threshold set at 0.05 ($p < 0.05$).

2.2.3. Method for Assessing the Dermal Irritant Effect

This study was conducted at the National Centre of Expertise in the Akmola region, which is accredited to perform toxicological assessments of plastic products on test subjects. Each experimental group consisted of 10 animals, adhering to the state standards that require a minimum of 6 animals per group [86]. Applications were made on the skin of rabbits, covering at least 5% of the total skin surface, which corresponded to an area of 7×8 cm^2.

A day prior to the experiment, the fur of the animals was carefully clipped using an electric trimmer on symmetrical areas of the back, leaving 1–2 cm of fur between the clipped sections. The right side of the back was designated for the application of the test substance, while the left side served as the control. To prevent the animals from licking the applied substance and to minimize inhalation, they were housed individually in specialized enclosures during the exposure period.

Gauze pads soaked in a water extract of microplastics were applied to the skin of the rabbits. The preparation of these extracts involved adding 1 g of the specific type and fraction of microplastic to 100 cm^3 of distilled water at room temperature (18–24 °C, extraction duration—4 days under thermostatic conditions, exposure duration—4 h). Clinical manifestations of intoxication and the condition of the skin were monitored 1 h and 24 h after application and subsequent washing of the substance. The presence of a skin-intoxication effect was evaluated based on indicators of skin functional disorders, including erythema, edema, fissures, ulcers, and changes in the temperature and neutralizing capacity of the skin. The experiment was conducted in triplicate.

2.2.4. Method of Acute Toxicity Study Based on Frozen Bovine Semen

This study was conducted at TEKS LLP, which is accredited to perform toxicological assessments of polymeric materials in accordance with the Methodological Guidelines [87]. The method employed involves examining changes in sperm motility in response to chemical compounds present in the extracts derived from the samples under investigation. The motility index is determined by measuring changes in light intensity as spermatozoa move through an optical probe.

The preparation of aqueous extracts was done as follows. For testing, 30 g of each microplastic fraction (PS, PET, PP, and PE) were prepared. Using a quartering method, 1 g suspensions microplastic were extracted from each fraction and placed in heat-resistant flasks with a capacity of 250 cm^3. A total of 100 cm^3 of boiling distilled water was added, and the flasks were incubated in a thermostat at a temperature of (40 ± 2) °C for 24 h. To establish a toxicity index, the experimental solutions were compared against a control (model) medium. Glucose-citrate medium (glucose—4 g, sodium citrate—1 g, and distilled water—100 cm^3) was selected as the control solution, which also served as the diluent for thawing frozen semen.

The experimental solution consisted of an aqueous extract of microplastics adjusted to isotonicity using dry reagents of glucose and sodium citrate (glucose—4 g, sodium citrate—1 g, and test solution—100 cm^3). Control and experimental solutions of 0.4 cm^3 each were placed in test tubes with lapped plugs and incubated in a water bath at (40 ± 1.5) °C. Both the control and experimental solutions were prepared one hour before the experiment. Subsequently, 0.1 cm^3 of semen stock solution was added to each test tube of the control and experimental series. Each working sample was then transferred into a cuvette, sealed, placed in a hood test stand, and tested immediately for 10–300 s [87].

The test results were evaluated by comparing the sperm motility of bovine semen from the tested samples to that of the control sample, which was designated as 100% (toxicity index). The experiment was repeated until the standard deviation of three parallel tests did not exceed 1%.

2.2.5. Method for Analyzing Phenol and Formaldehyde Migration Values

This study was conducted in accordance with [88]. This normative document establishes the values of PAM (mg/dm^3)—the permissible amount of migration of a chemical substance—as a limiting indicator. Sanitary–hygienic requirements are set for formaldehyde migration in polymeric particles such as PS, PET, PP, and PE. While these polymers are not typically evaluated for phenol migration due to their chemical structures, we opted to include phenol migration assessment in this experiment to evaluate the quality of the tested products.

The experiment was carried out using distilled water under the following conditions: temperature—22–25 °C and exposure time extraction duration—3 h under thermostatic conditions [88]. To ensure that standardized conditions [88] were met (the ratio of the microplastic particle area to the water surface area should be no less than 1 cm^2:2 cm^2) and that the condition of uniform microplastic concentration in the extracted solutions was met in all our experiments, 1 g of microplastic was taken and 100 cm^3 of distilled water at a temperature of 22–25 °C was added to obtain the extracts.

The concentration of formaldehyde was determined using a photocolorimetric method with a Jenway 6320D spectrophotometer (Jenway Ltd., Gransmore Green, Great Britain), with a measurement accuracy of ±0.01 mg/dm^3, in accordance with [89]. The phenol concentration was assessed via gas chromatography using a "Chromos GC-1000" gas chromatograph equipped with a flame ionization detector (Chromos Ltd., Dzerzhinsk, Russia), also with a measurement accuracy of ±0.01 mg/dm^3 [86].

The concentrations of phenol and formaldehyde in the aqueous extracts were then compared against the sanitary norms for the permissible migration quantities (PMQs) of these substances.

3. Results and Discussion

3.1. Assessment of Phytotoxic Properties of Microplastics

When barley seeds were soaked in the aqueous extracts of all studied fractions of microplastics, a deterioration in the physiological parameters of the barley was observed compared to the negative control (Table 1). Additionally, after 2–3 days, rotting formations were noted in the majority of seeds treated with the aqueous extracts of all types of the studied microplastics.

Table 1. Percentage of germination and speed, friendliness of seed germination when soaking seeds with water extract of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE).

Experiment Variant		Germination, %	Germination Rate, Conventional Units	Germination Density, %
Negative control, dHO_2		86.67 ± 1.76	39.39 ± 0.81	28.89 ± 0.59
Positive control, MMC (5 mg/dm)^3		66.67 ± 1.76 **	31.39 ± 1.19 *	22.22 ± 0.72 **
Polystyrene (PS)	0.175 mm	69.33 ± 3.53 *	30.83 ± 1.30 *	23.11 ± 1.18 *
	0.3 mm	71.3300 ± 3.53	32.83 ± 1.78	23.78 ± 1.18
	1.0 mm	72.00 ± 4.16	29.61 ± 2.14 *	24.00 ± 1.39
	2.0 mm	74.00 ± 3.46	31.89 ± 2.50	24.67 ± 1.15
	3.0 mm	75.33 ± 4.37	33.39 ± 2.31	25.11 ± 1.46
Polyethylene terephthalate (PET)	0.175 mm	73.33 ± 1.76 *	33.72 ± 0.86 *	24.44 ± 0.59 *
	0.3 mm	76.67 ± 3.33	33.72 ± 1.33	25.56 ± 1.11
	1.0 mm	77.33 ± 5.46	34.56 ± 1.53	25.78 ± 1.82
	2.0 mm	78,00 ± 4.62	36.72 ± 0.70	26.00 ± 1.54
	3.0 mm	78.67 ± 2.40	35.61 ± 0.87	26.22 ± 0.80
Polypropylene (PP)	0.175 mm	72.67 ± 2.40 *	33.17 ± 1.42 *	24.22 ± 0.80 *
	0.3 mm	74.67 ± 2.40 *	32.67 ± 0.75 **	24.89 ± 0.80 *
	1.0 mm	76.00 ± 4.00	35.89 ± 2.12	25.33 ± 1.33
	2.0 mm	77.33 ± 2.91	35.22 ± 1.27	25.78 ± 0.97
	3.0 mm	79.33 ± 4.06	34.06 ± 0.48 **	26.44 ± 1.35
Polyethylene (PE)	0.175 mm	69.33 ± 2.91 *	28.83 ± 2.18 *	23.11 ± 0.97 *
	0.3 mm	70.00 ± 3.06 *	31.17 ± 1.04 *	23.33 ± 1.02 *
	1.0 mm	71.33 ± 3.71	30.89 ± 1.65 *	23.78 ± 1.24
	2.0 mm	72.67 ± 3.53	32.28 ± 1.26 *	24.22 ± 1.18
	3.0 mm	75.33 ± 2.91	33.22 ± 0.96 *	25.11 ± 0.97

Note: * $p < 0.05$, ** $p < 0.01$—compared to negative control.

Specifically, when seeds were exposed to polystyrene (PS) microplastic of 0.175 mm size, there was a statistically significant decrease ($p < 0.05$) in seed germination, which was reduced by 1.25 times, along with a 1.28 times decrease in germination rate and a 1.25 times decrease in germination uniformity compared to the negative control. Notably, these decreases in physiological parameters were comparable to those observed in the positive control.

In seeds exposed to PS microplastic particles of sizes 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm, an increase in phytotoxic activity was observed; however, these changes were not statistically significant compared to the negative control. An exception was noted with the germination rate of barley seeds influenced by the 1.0 mm fraction of PS microplastic, which showed a statistically significant decrease of 1.33 times ($p < 0.05$).

When seeds were exposed to PET microplastic of 0.175 mm size, a statistically significant decrease in seed germination was observed, reduced by 1.18 times ($p < 0.05$), along with a 1.17 times decrease in germination rate and a 1.18 times decrease in germination uniformity compared to the negative control. This decrease in phytotoxicity indicators was comparable to the positive control.

For seeds exposed to PET microplastic sizes of 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm, a decrease in physiological indicators was also noted; however, these changes were not statistically significant compared to the negative control.

In the case of water extracts from PP microplastic fractions of 0.175 mm and 0.3 mm, statistically significant decreases in seed germination were observed, reduced by 1.19 times ($p < 0.05$) and 1.16 times ($p < 0.05$), respectively. Germination rates also decreased significantly by 1.19 times ($p < 0.05$) and 1.21 times ($p < 0.01$) for these fractions, respectively, as well as germination uniformity decreasing by 1.19 times ($p < 0.05$) and 1.16 times ($p < 0.05$). Notably, when seeds were exposed to PP microplastic of 3.0 mm size, a statistically significant decrease in the germination rate of 1.16 times ($p < 0.01$) compared to the negative control was observed. This decline in physiological parameters and the increase in phytotoxic activity of the PP microplastic was at a level similar to that of the positive control. Deterioration in the physiological indicators of barley seeds exposed to PP microplastic fractions of 1.0 mm and 2.0 mm was also observed, although these changes were not statistically significant compared to the negative control.

When seeds were exposed to microplastic PE fractions of 0.175 mm and 0.3 mm, statistically significant decreases in seed germination were observed, reduced by 1.25 times ($p < 0.05$) and 1.24 times ($p < 0.05$), respectively. Similar reductions were noted in the germination rates, which decreased by 1.25 times for the 0.175 mm fraction and 1.24 times for the 0.3 mm fraction compared to the negative control.

Additionally, significant decreases in germination rates were observed for all PE microplastic fractions compared to the negative control: 0.175 mm—1.37 times, 0.3 mm—1.26 times, 1.0 mm—1.28 times, 2.0 mm—1.22 times, and 3.0 mm—1.19 times. The deterioration of the barley's physiological parameters in these cases was similar to that observed in the positive control.

Statistical analysis did not reveal significant differences between the various types of microplastics tested. Thus, the data indicate that all four types of microplastics (PS, PET, PP, and PE) exhibited statistically significant phytotoxic properties, particularly for the smallest fraction studied (0.175 mm). For the 0.3 mm fraction, significant reductions in all studied physiological parameters of barley were found for PP and PE; for the 1.0 mm fraction, significant effects were noted for PS; for the 2.0 mm fraction, significant effects were observed for PE; and for the 3.0 mm fraction, significant effects were noted for both PP and PE.

Phytotoxic activity manifested as growth-inhibiting effects on germinating barley seeds, along with the presence of rot formations within the seeds.

A few studies on the phytotoxicity of microplastics have indicated negative effects on seed growth due to toxicants leached from plastics [43]. These studies also suggest that the effects of plastics can vary based on concentration, polymer type, and plant species [90,91].

Furthermore, nanosized plastic particles may exert physical blocking effects, clogging pores and preventing water entry, thereby inhibiting seed growth [44].

3.2. Assessment of Chromosomal Abnormalities

When *H. vulgare* seeds were exposed to aqueous extracts of all types of the studied microplastics, an increase in the frequency of chromosomal abnormalities in apical meristem cells was observed compared to the baseline levels of spontaneous mutations. Table 2 presents the data on the frequency of chromosomal aberrations in the meristems of common barley germinal roots that were soaked in aqueous extracts of microplastics from polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE).

Table 2. Frequency of chromosomal abnormalities in common barley root meristem cells germinated on aqueous extracts of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) microplastics.

Experiment Variant		Total Cells	Frequency of Aberrant Cells (M ± m,%)	Number of Chromosomal Aberrations per 100 Metaphase Cells		
				Total Aberrations	Chromosomal Type	Chromatid Type
Negative control, dHO_2		450	1.11 ± 0.49	1.11 ± 0.49	0.44 ± 0.31	0.67 ± 0.38
Positive control, MMC (5 mg/dm)^3		471	5.31 ± 1.03 ***	5.94 ± 1.09 ***	2.55 ± 0.73 **	3.40 ± 0.83 **
Polystyrene (PS)	0.175 mm	480	3.75 ± 0.87 **	4.58 ± 0.95 **	2.08 ± 0.65 *	2.50 ± 0.71 *
	0.3 mm	511	3.52 ± 0.82 *	3.91 ± 0.86 **	1.37 ± 0.51	2.54 ± 0.70 *
	1.0 mm	512	3.32 ± 0.79 *	3.91 ± 0.86 **	1.56 ± 0.55	2.34 ± 0.67 *
	2.0 mm	458	2.84 ± 0.78	3.28 ± 0.83 *	1.09 ± 0.49	2.18 ± 0.68
	3.0 mm	446	2.69 ± 0.77	2.91 ± 0.80	0.90 ± 0.45	2.02 ± 0.67
Polyethylene Terephthalate (PET)	0.175 mm	462	4.55 ± 0.97 **	5.41 ± 1.05 ***	2.16 ± 0.68 *	3.25 ± 0.82 **
	0.3 mm	482	3.94 ± 0.89 **	4.15 ± 0.91 **	1.87 ± 0.62 *	2.28 ± 0.68 *
	1.0 mm	425	4.24 ± 0.98 **	4.71 ± 1.03 **	1.18 ± 0.52	3.53 ± 0.90 **
	2.0 mm	436	3.67 ± 0.90 *	3.90 ± 0.93 **	1.61 ± 0.60	2.29 ± 0.72 *
	3.0 mm	440	3.64 ± 0.89 *	3.86 ± 0.92 **	1.59 ± 0.60	2.27 ± 0.71 *
Polypropylene (PP)	0.175 mm	500	3.20 ± 0.79 *	3.40 ± 0.81 *	1.60 ± 0.56	1.80 ± 0.59
	0.3 mm	490	3.06 ± 0.78 *	3.06 ± 0.78 *	1.43 ± 0.54	1.63 ± 0.57
	1.0 mm	442	2.94 ± 0.80	2.94 ± 0.80	1.36 ± 0.55	1.58 ± 0.59
	2.0 mm	456	2.85 ± 0.78	2.85 ± 0.78	1.32 ± 0.53	1.54 ± 0.58
	3.0 mm	495	2.63 ± 0.72	2.63 ± 0.72	1.21 ± 0.49	1.41 ± 0.53
Polyethylene (PE)	0.175 mm	495	3.23 ± 0.79 *	3.64 ± 0.84 *	1.82 ± 0.60 *	1.82 ± 0.60
	0.3 mm	500	3.20 ± 0.79 *	3.40 ± 0.81 *	1.60 ± 0.56	1.80 ± 0.59
	1.0 mm	520	2.88 ± 0.73 *	2.88 ± 0.73 *	1.15 ± 0.47	1.73 ± 0.57
	2.0 mm	465	2.80 ± 0.76	2.80 ± 0.76	1.08 ± 0.48	1.72 ± 0.60
	3.0 mm	458	2.40 ± 0.72	2.40 ± 0.72	0.87 ± 0.43	1.53 ± 0.57

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to negative control.

The frequency of aberrant cells exposed to PS microplastic fractions of 0.175 mm, 0.3 mm, and 1.0 mm significantly increased compared to the negative control, by 3.38-fold ($p < 0.01$), 3.17-fold ($p < 0.05$), and 2.99-fold ($p < 0.05$), respectively. For PS microplastic fractions of 2.0 mm and 3.0 mm, the frequency of aberrant cells also increased compared to the negative control, by 2.56-fold and 2.42-fold, respectively; however, these increases were not statistically significant.

When assessing the number of chromosomal aberrations per 100 metaphases in the apical meristem of barley seeds exposed to aqueous extracts of microplastics from PS fractions, statistically significant increases were observed compared to the negative control. Specifically, exposure to the aqueous extract of the 0.175 mm PS fraction resulted in a

4.13-fold increase ($p < 0.01$) in chromosomal aberrations. The number of chromosomal and chromatid aberrations increased 4.73-fold ($p < 0.05$) and 3.73-fold ($p < 0.05$), respectively.

For the 0.3 mm PS fraction, the number of chromosomal structural rearrangements per 100 metaphases increased by 3.52-fold ($p < 0.01$) compared to the negative control, while chromosomal and chromatid aberrations increased 3.11-fold and 3.79-fold ($p < 0.05$), respectively. Similarly, exposure to the aqueous extract of the 1.0 mm PS fraction led to a 3.52-fold increase in chromosomal aberrations ($p < 0.01$), with chromosomal and chromatid aberrations increasing 3.54-fold and 3.49-fold ($p < 0.05$), respectively.

For the 2.0 mm PS fraction, a statistically significant increase in chromosomal aberrations was observed, with a 2.95-fold increase ($p < 0.05$) compared to the negative control. The number of chromosomal and chromatid aberrations increased by 2.48-fold and 3.01-fold, respectively. Exposure to the aqueous extract of the 3.0 mm PS fraction also resulted in a significant increase in chromosomal aberrations, with a 2.62-fold increase compared to the negative control and a 2.05-fold and 3.01-fold increase in chromosomal and chromatid aberrations, respectively.

When barley seeds were exposed to aqueous extracts of PET microplastic of the 0.175 mm fraction, a statistically significant increase in the frequency of aberrant cells was observed, with a 4.10-fold increase ($p < 0.01$) compared to the negative control. The number of chromosomal structural rearrangements per 100 metaphases also significantly increased by 4.87-fold ($p < 0.001$). Additionally, the number of chromosome-type aberrations increased 4.91-fold ($p < 0.05$) and chromatid-type aberrations increased 4.85-fold ($p < 0.01$).

For the 0.3 mm PET fraction, the frequency of aberrant cells significantly increased by 3.55-fold ($p < 0.01$) compared to the negative control, while the number of chromosomal rearrangements per 100 metaphases increased by 3.74-fold ($p < 0.01$). The number of chromosomal type aberrations increased 4.25-fold ($p < 0.05$) and the number of chromatid type aberrations increased 3.40-fold ($p < 0.05$).

Exposure to aqueous extracts of the 1.0 mm PET fraction resulted in a statistically significant increase in the frequency of cells with chromosomal aberrations, with a 3.82-fold increase ($p < 0.01$), and a 4.24-fold increase ($p < 0.01$) in the number of chromosomal aberrations per 100 metaphases compared to the negative control. This fraction also exhibited a significant increase in chromosomal type rearrangements (2.68-fold) and chromatid type rearrangements (5.27-fold, $p < 0.01$).

The 2.0 mm PET fraction caused a statistically significant increase in the frequency of cells with chromosomal aberrations, by 3.31-fold ($p < 0.05$), and in the number of chromosomal rearrangements per 100 cells, by 3.51-fold ($p < 0.01$), compared to the negative control. The number of chromosome-type aberrations increased 3.66-fold and chromatid-type aberrations increased 3.42-fold ($p < 0.05$).

Finally, exposure to aqueous extracts of the 3.0 mm PET fraction resulted in a statistically significant increase in the frequency of cells with chromosomal structural abnormalities, with a 3.28-fold increase ($p < 0.05$) compared to the negative control. The number of chromosomal aberrations per 100 metaphases increased by 3.48-fold ($p < 0.01$), while chromosome-type aberrations increased 3.61-fold and chromatid-type aberrations increased 3.39-fold ($p < 0.05$).

When barley seeds were exposed to aqueous extracts of PP microplastic, the frequency of aberrant cells significantly increased compared to the negative control. For the 0.175 mm and 0.3 mm fractions, the frequency of aberrant cells increased by 2.88-fold ($p < 0.05$) and 2.76-fold ($p < 0.05$), respectively. In contrast, the frequencies of aberrant cells for the 1.0 mm, 2.0 mm, and 3.0 mm fractions increased by 2.65-fold, 2.57-fold, and 2.37-fold compared to the negative control, but these increases were not statistically significant.

When evaluating the number of chromosomal aberrations per 100 metaphases in the apical meristem of barley seeds, significant increases were noted for the 0.175 mm and 0.3 mm fractions compared to the negative control. Specifically, exposure to the aqueous extracts of the 0.175 mm PP fraction resulted in a 3.06-fold increase ($p < 0.05$)

in the number of chromosomal aberrations. Additionally, chromosomal and chromatid aberrations increased by 3.64-fold and 2.69-fold, respectively.

For the 0.3 mm PP fraction, the increase in the number of structural rearrangements of chromosomes per 100 metaphases was 2.76-fold ($p < 0.05$) compared to the negative control, with chromosomal and chromatid aberrations increasing by 3.25-fold and 2.43-fold, respectively.

When barley seeds were exposed to aqueous extracts of the 1.0 mm PP fraction, there was a 2.65-fold increase in the number of chromosomal aberrations per 100 metaphases compared to the negative control. This exposure also led to increases of 3.09-fold and 2.36-fold in chromosomal and chromatid aberrations, respectively.

For the 2.0 mm PP fraction, the number of chromosomal aberrations per 100 metaphases increased by 2.57-fold compared to the negative control, with chromosomal and chromatid aberrations rising by 3.00-fold and 2.30-fold, respectively. Finally, exposure to the aqueous extract of the 3.0 mm PP fraction resulted in a 2.37-fold increase in chromosomal aberrations per 100 metaphases compared to the negative control, with chromosomal and chromatid aberrations increasing by 2.75-fold and 2.10-fold, respectively.

When barley seeds were exposed to aqueous extracts from PE microplastic of the 0.175 mm fraction, a statistically significant increase in the frequency of aberrant cells was observed, rising 2.91 times ($p < 0.05$) compared to the negative control. The number of chromosomal structural rearrangements per 100 metaphases also increased by 3.28 times ($p < 0.05$). Additionally, chromosomal type aberrations increased by 4.14 times ($p < 0.05$), while chromatid type aberrations rose by 2.72 times.

When exposed to aqueous extracts from the 0.3 mm PE microplastic fraction, the frequency of aberrant cells increased significantly by 2.88 times ($p < 0.05$) compared to the negative control and the number of chromosomal rearrangements per 100 metaphases increased by 3.06 times ($p < 0.05$). The number of chromosomal type aberrations increased by 3.64 times, while chromatid type aberrations rose by 2.69 times.

For the 1.0 mm PE microplastic fraction, a statistically significant increase in the level of cells with chromosomal aberrations was noted, increasing by 2.59 times ($p < 0.05$). The number of chromosomal aberrations per 100 metaphases also rose by 2.59 times ($p < 0.05$). Furthermore, chromosomal type rearrangements increased by 2.61-fold and chromatid type rearrangements increased by 2.58-fold.

The 2.0 mm PE microplastic fraction caused an increase in the frequency of cells with chromosomal aberrations by 2.52 times and the number of chromosomal rearrangements per 100 cells also increased by 2.52 times compared to distilled water. However, while the number of chromosomal type aberrations increased by 2.45-fold and the number of chromatid-type aberrations increased by 2.57-fold, these increases were not statistically significant.

A similar trend was observed with the 3.0 mm PE microplastic fraction. The frequency of cells with chromosomal structural abnormalities increased by 2.16 times compared to the negative control, as did the number of chromosomal aberrations per 100 metaphases. The number of chromosomal aberrations rose by 1.97 times and chromatid aberrations increased by 2.28 times; however, these increases were not statistically significant. In the studied cells of the barley apical meristem under the influence of various microplastics, structural disorders of chromosomes were noted at the metaphase stage, irrespective of the type (PS, PET, PP, and PE) or size of the microplastic (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm). Observed abnormalities included single and paired deletions, centric rings, point fragments, and instances of polyploidy. Anaphase stage abnormalities included chromosome lagging and chromosome bridges (see Figure 2).

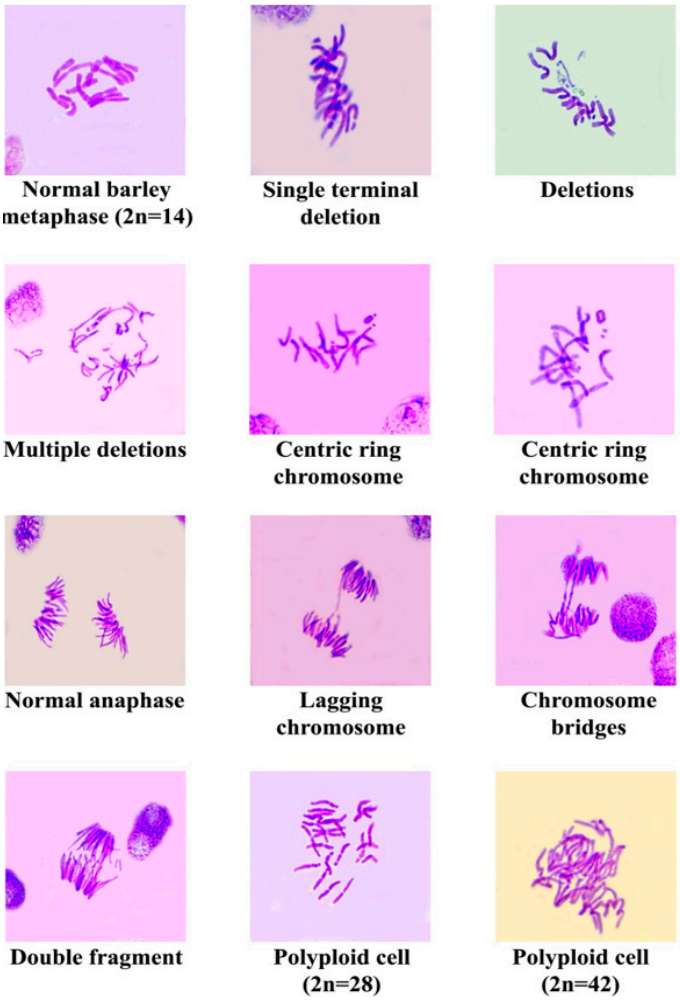

Figure 2. Chromosomal and genomic abnormalities in root germ meristem cells of *Hordeum vulgare* L. induced by PS, PET, PP, and PE of fractions 0.175 mm, 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm.

The results of the study on the genotoxicity of aqueous extracts from microplastics demonstrated a statistically significant increase in both the frequency of aberrant cells and the number of chromosomal aberrations in the apical meristem cells of barley. All types of microplastics studied—PS, PET, PP, and PE—exhibited mutagenic effects, with these effects being most pronounced for smaller fractions. The average increases in the frequency of aberrant cells and the number of chromosomal aberrations per 100 metaphases were as follows: for the 0.175 mm fraction, 3.32 and 3.84 times; for the 0.3 mm fraction, 3.09 and 3.27 times; and for the 1 mm fraction, 3.06 and 3.23 times, respectively. For the 2 mm and 3 mm fractions, statistically significant genotoxicity was observed only for PET.

The spectrum of chromosomal aberrations was broad and independent of the type and size of microplastic. It included deletions, multiple breaks, centric rings, polyploids, chromosomal bridges, and lagging chromosomes during anaphase. The order of microplastic types based on decreasing genotoxicity was as follows: PET > PS > PE > PP.

This increase in genotoxicity with smaller particle sizes may be attributed to the enhanced dispersibility of microplastics, which leads to an increased surface area for extractable substances to migrate into aqueous solutions (solvents, monomers, etc.). These extracted chemicals can impart toxicity to the aqueous extracts of microplastics and exhibit phytotoxic effects and mutagenic activity in the presence of inert particles [13,14]. Similar unexplained genotoxic effects, which intensified with decreasing particle size, were also reported in experiments involving polystyrene [92].

In contrast, the study of the dermal irritant properties of aqueous extracts from all investigated types of microplastics (PS, PET, PP, and PE) across all fractional sizes revealed no significant changes to the skin compared to the control group. This suggests that a single application of aqueous extracts obtained at room temperature for a short duration (1 day) does not reveal toxic properties of the microplastics. However, further research is necessary, involving repeated exposure to microplastic extracts over extended extraction times (up to 30 days), varying the temperature of distilled water used for the extraction, and simulating conditions that mimic the use of plastics in a domestic setting.

3.3. Results of Acute Toxicity Studies on Frozen Bull Semen

The results of the experiments are presented in Table 3.

Table 3. Toxicity index of aqueous fractions of microplastics established in the experiment on frozen bull semen.

Polymer	Fraction, mm	Toxicity Index, %	Deviation of Sperm Motility of Bull Semen from the Norm, %
Polystyrene (PS)	0.175	94.2	5.8
	0.3	94.0	6.0
	1.0	92.4	7.6
	2.0	93.0	7.0
	3.0	93.8	6.2
The average deviation of the PS is 6.52%			
Polyethylene terephthalate (PET)	0.175	94.2	5.2
	0.3	93.1	6.9
	1.0	96.7	3.3
	2.0	99.4	0.6
	3.0	90.9	9.1
The average deviation on PET is 5.02%			
Polypropylene (PP)	0.175	98.7	0.3
	0.3	99.8	0.2
	1.0	105.6	5.6
	2.0	109.9	9.9
	3.0	107.2	7.2
The average deviation on PP is 4.64%			
Polyethylene (PE)	0.175	97.9	2.1
	0.3	103.7	3.7
	1.0	96.8	3.2
	2.0	101.3	1.3
	3.0	99.5	0.5
The average deviation in PE is 2.16%			

As a result of the experiment, no significant effect of microplastic fractional size within the range of 0.175 to 3 mm on the motility of bovine spermatozoa was observed. However, an increase in toxicity was noted with greater complexity in the polymer structure. The smallest deviation in sperm motility from the control was recorded for polyethylene (PE), which has a simple structure characterized by a straight chain of methylene groups (CH_2). In contrast, polypropylene (PP) has a more complex structure due to the presence of a methyl group in the side chain, resulting in a 2.16% reduction in sperm motility.

Polyethylene terephthalate (PET) features a benzene ring (C_6H_4) in its main chain, which not only enhances the rigidity of the PET macromolecule but also elevates its glass transition temperature and melting point. This structural complexity appears to influence its toxic properties. According to the experimental results, the reduction in sperm motility for PET microplastic particles was 2.32% compared to PE and 8.19% compared to PP.

In contrast, polystyrene (PS) has a benzene ring located in its side chain, which contributes to its brittleness and likely impacts its toxic properties adversely. In our experiment, the change in sperm motility for PS was 1.3% lower than for PET and 3.02% lower than for PE.

It is hypothesized that the experimental conditions—including treatment with boiled water and daily thermostatting at elevated temperatures (40 ± 1.5 °C)—may facilitate the formation of toxic organic compounds such as bisphenol A (BPA) [93,94]. BPA is a known toxicant with detrimental effects on the reproductive system [95,96]. This concern is particularly relevant for polymers with chemical structures that include benzene rings. However, these assumptions warrant further investigation [97].

3.4. Results of Phenol and Formaldehyde Migration Analyses

The results of the experiment are presented in Table 4.

Table 4. Migration of phenol and formaldehyde into aqueous extracts from microplastic fractions of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) microplastics.

Polymer	Fraction (mm)	Phenol (mg/dm^3)/PAM Share Not More than 0.05	Formaldehyde (mg/dm^3)/PAM Share Not More than 0.1
PAM			
Polystyrene (PS)	0.175	<0.01/0.02	0.30/3.0
	0.3	<0.01/0.02	0.40/4.0
	1.0	<0.01/0.02	0.31/3.1
	2.0	<0.01/0.02	0.11/1.1
	3.0	<0.01/0.02	0.12/1.2
Polyethylene terephthalate (PET)	0.175	<0.01/0.02	1.04/10.4
	0.3	<0.01/0.02	0.82/8.2
	1.0	<0.01/0.02	0.50/5.0
	2.0	<0.01/0.02	0.61/6.1
	3.0	<0.01/0.02	0.40/4.0
Polypropylene (PP)	0.175	<0.01/0.02	1.04/10.4
	0.3	<0.01/0.02	1.21/12.1
	1.0	<0.01/0.02	0.30/3.0
	2.0	<0.01/0.02	0.35/3.5
	3.0	<0.01/0.02	0.26/2.6
Polyethylene (PE)	0.175	<0.01/0.02	0.41/4.1
	0.3	<0.01/0.02	0.40/4.0
	1.0	<0.01/0.02	0.34/3.4
	2.0	<0.01/0.02	0.30/3.0
	3.0	<0.01/0.02	0.32/3.2

As shown in Table 4, the migration of phenol into the aqueous extract is observed at levels five times lower than the permissible value for dichloromethane (PAM). In contrast, the concentration of formaldehyde in the water extracts exceeds the PAM limit by factors ranging from 1.1 to 12.1, depending on the type and fraction of microplastic. The highest levels of formaldehyde migration are detected in polypropylene (PP), which we selected as a representative of water pipe material (averaging 6.32 PAM for all fractions), and in polyethylene terephthalate (PET), chosen as a sample of disposable plastic bottles (averaging 6.74 PAM across all fractions).

Notably, the migration of formaldehyde increases as the size of the microplastic fraction decreases. Therefore, it can be concluded that using water pipes made from polyethylene (PE) and PP, as well as disposable tableware crafted from PET and polystyrene (PS), even at room temperature and after a short exposure period (3 h), may result in formaldehyde migration that surpasses the permissible sanitary standards of the Eurasian Economic Union (EurAsEU).

Furthermore, the use of water pipes made from PP and PE with hot water, and disposable containers made from PET and PS for hot products, may lead to significantly higher levels of formaldehyde migration. This warrants additional research in both aqueous and gaseous environments.

Thus, a comparative analysis of published sources (see Table 5) showed that a number of studies of the toxicity of micro- and nanoplastics on plant test systems (seed germination, growth, and development of plant test objects) were carried out in soil, which, unlike in our experiment and some other studies on plant seeds, allows for climatic factors, soil quality, and microbial community to influence the results obtained [98].

Table 5. Comparative analysis of published sources.

Type of Microplastic (MP or NP)	Concentration	Origin of MP	Object and Studied Indicators	Research Result	Source
PP-MP PE-MP PS-MP	1 g/100 dm^3 of water (extractant, for seeds)	Hand-crushed to resemble natural form	Barley *Hordeum vulgare* L.: seed growth indicators, genotoxicity in apical meristem cells Acute toxicity to bull semen Formaldehyde migration Phenol migration Skin irritant effect on the dermis of rabbits	decreased germination rates, increased frequency of aberrant cells and number of chromosomal aberrations presence of influence on sperm motility exceeding permissible levels of migration no toxic effects detected no toxic effects detected	This study
PS-MP PS-NP	10^{-3}–10^{-7} particles/sm^3 extractant (water)	spherical (standardized production)	Watercress *Lepidium sativum:* seed germination, root and shoot growth, chlorophyll content, seed growth indicators	significant effect on seed germination and root growth on the first day, the adverse effect increased with the transition from NP to MP	[99]
PET-MP PVC-MP	0.5% (by soil weight)	spherical (standardized production)	Tomato *Solanum lycopersicum* L.: growth and number of fruits	Negative effects on growth and physiology, fruit set; increased anti-nutritional properties	[100]
LDPE-MP	0.4% (by soil weight) in a climate-controlled chamber	Hand-crushed	Wheat Triticum aestivum: Wheat growth exposed to low-density polyethylene (LDPE) and biodegradable starch-PET plastics	Negative impact on aboveground and underground parts of the plant during vegetative and reproductive growth	[101]
MP: PET, PA, PP, PE, PU, PS, PC, PES	0.4% (by soil weight)	Hand-chopped	Wild carrot *Daucus carota:* seed germination	Deterioration of seed germination rates depending on MPs shape	[102]
PE-MP	0.25, 0.50, 0.75 and 1.0% of water (extractant, for seeds)	Fragments (industrial production)	Blackgram (*Vigna mungo* L.) and tomato *Solanum lycopersicum* L.): growth and physiological parameters	effect on seed germination, root length and shoots, depending on the dose, type and duration of exposure	[91]
PS (MP, NP)	2 µm–80 nm in 0, 10, 50, 100 and 500 mg/L of water (extractant, for plants in the soil)	Fragments (industrial production)	Ornamental plants: *Trifolium repens*, *Orychophragmus violaceus* and Impatiens balsamina: seed germination tendency, germination rate and various physiological and biochemical parameters	inhibitory effect on seed germination processes	[103]
PMMA (MP, NP)	0, 0.01, 0.1, 1, 5 y 10 g/L (for plants, on soils)	Fragments (industrial production)	Rape *Brassia campestris* L.: Single and combined effects	Suppression of growth index GI, biomass growth biomass, root length and shoot length	[104]

Table 5. *Cont.*

Type of Microplastic (MP or NP)	Concentration	Origin of MP	Object and Studied Indicators	Research Result	Source
HDPE Mater-bi®, MB пакеты	pieces of plastic bags of size approximately 1 cm^2, in liquid (water) to solid (plastic) ratios of 100, 10 and 5, corresponding to approximately 4.1×10^{-4}, 4.1×10^{-3} and 8.3×10^{-3} bag/mL respectively (extractant, for seeds and seedlings)	Hand-crushed to resemble natural form	Garden cress *Lepidium sativum* L: seed germination	developmental abnormalities and decreased plant growth	[43]
PES-MP PP-MP	0.4% (by soil weight)	Fragments (industrial production)	*Corn Zea mays*, *Soybean Glycine max*, *Peanut Arachis hypogaea*: growth, physiological and biochemical parameters	negative consequences for plant growth, biomass accumulation and its quality	[105]
PS-NP	200 nm 0.1–1000 mg/L (for plants in the soil)	Fragments (industrial production)	Rice seeds: seed germination, root growth, antioxidant enzyme activity and transcriptome	gene expression, changes in growth rates, root length, accumulation of reactive oxygen species in the roots. No significant effect on seed germination was found. Significant increase in root length and decrease in antioxidant enzyme activity	[45]
PET-MP PET-NP	0.02% (*w*/*w* microplastic/soil)	industrial pellet crushing	Watercress *Lepidium sativu:* seed germination, plant height, fresh biomass production, oxidative stress response, photosynthetic apparatus disruption, aminolevulinic acid and proline production	The percentage of inhibition of seed germination was the only parameter that showed statistically significant changes	[106]
PS-MP	0.6–0.7 µg/day, 6–7 µg/ day, 60–70 µg/da	spherical (standardized production)	Mice	Decreased sperm quality, abnormal testicular spermatogenesis	[107]
PS-NP	50 mg/kg/day orally	spherical (standardized production)	Mice	Decreased fertility, expression of genes associated with apoptosis and inflammation	[108]

Only in the work [101] was it possible to ensure that climatic factors did not influence the growth of wheat seeds, but the influence of soil parameters remained. Various crops have been chosen as plant test objects, such as cress salad [43,99,106], ornamental plants [103] and wild carrot [102]. Of the agricultural crops, the following plant species were studied that are most typical for the study region: barley (our study), tomatoes and fruits [100], wheat [101], blackgram and tomatoes [91], rapeseed [104], corn, soybean, peanut [105], and rice [45]. Most studies have examined the effects of microplastics on growth, biochemical, and productivity characteristics of plants. However, only a few studies have focused on the effects of microplastic toxicity on seed germination, a critical stage in the plant life cycle. All studies confirm the negative impact of plastic particles on seed germination [103], as established in our experiment, depending on the dose, type of plastics, and duration of exposure, increasing the negative impact on seed germination and root growth in the first day when switching from microplastics to nanoplastics [99], which justifies our choice of the size of the studied particles. The importance of studying seed germination when studying the toxicity of micro- and nanoplastics is confirmed by

the study [106]. Among the parameters studied in the study (seed germination, plant height, fresh biomass production, oxidative stress response, photosynthetic apparatus impairment, and aminolevulinic acid and proline production), the percentage of seed germination inhibition was the only parameter that showed statistically significant changes. In the work [104], inhibition of root growth was observed along with suppression of the growth index GI, biomass growth, and shoot length. One of the studies included the results of a genetic test, which determined gene expression as well as changes in physiological parameters—changes in growth rates, root length, and accumulation of active oxygen species in rice roots [45]. However, the authors did not find any significant effect of polystyrene nanoparticles on seed germination.

The effect of microplastic toxicity on sperm was studied in our experiment on frozen bull sperm, but a comparative assessment with other studies could not be carried out due to the lack of similar experiments. Most studies are conducted on animals (with oral administration), which is subject to the influence of other factors (diet, hunched position of the animals, etc.) and does not allow a clear assessment of the contribution of microplastic toxicity to the effects detected. However, such studies have found decreased sperm quality, abnormal testicular spermatogenesis, decreased fertility, and expression of genes associated with apoptosis and inflammation for microplastics [107] and nanoplastics [108].

The presence of contradictory research results, the organization of phytotoxicity and genotoxicity studies on individual types of microplastics (mostly represented by industrial samples of spherical particles, far from the real shape of micro- and nanoplastics in water), a small selection of plastic types (often polystyrene as an object of influence), and toxicity assessment only on single test objects, in contrast to the comprehensive approach in our case, make this study relevant and new.

4. Conclusions

The presence of microplastics (polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE)) in the tap water of Kokshetau city (Akmola region, Kazakhstan) has raised concerns regarding the potential toxic risks associated with microplastics in the region's drinking water and the possibility of skin irritation during bathing. Our study evaluated the toxicity of aqueous extracts of polymer particles fragmented to microplastic sizes rather than the polymers themselves. The investigation encompassed various microplastic fractions (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm), thus varying the contact surface area of microplastics with water and their extraction efficiency.

Recognizing the inadequacy of assessing the toxic properties of synthetic materials through a singular method, and to mitigate ethical concerns regarding human and extensive animal testing, we conducted a comprehensive evaluation of the toxicity of aqueous extracts of microplastics on both plant and animal test organisms, including frozen bull semen, alongside analyses of organic matter migration into the aquatic environment.

Germination experiments with *Hordeum vulgare* L. seeds revealed phytotoxic effects of water extracts from PS, PET, PP, and PE microplastics, particularly for the 0.175 mm fraction. The 0.3 mm fraction of PP and PE, the 1.0 mm fraction of PS, the 2.0 mm fraction of PE, and the 3.0 mm fractions of PP and PE significantly reduced various physiological indices of barley. Phytotoxicity manifested as inhibited growth and seed rot. No significant differences in phytotoxicity were observed among the different microplastic types.

Genotoxicity studies demonstrated a significant increase in the frequency of aberrant cells and chromosomal aberrations in barley apical meristem cells exposed to aqueous extracts of all microplastic types (PS, PET, PP, and PE), with smaller fractions exhibiting more pronounced effects. The 0.175 mm fraction increased the frequency of aberrant cells and chromosomal aberrations by 3.32 and 3.84 times, respectively, compared to the control. Similar increases were observed for the 0.3 mm and 1 mm fractions. PET microplastics, especially in the 2 mm and 3 mm fractions, also exhibited significant genotoxicity. The spectrum of chromosomal aberrations was broad, encompassing deletions, multiple breaks, centric rings, polyploidy, chromosomal bridges, and lagging chromosomes, and was in-

dependent of microplastic type or size. In terms of decreasing genotoxicity, the order was PET > PS > PE > PP.

No significant skin irritation was observed from aqueous extracts of any microplastic type or size. The assessment of bovine semen motility revealed increased toxicity with increasing polymer complexity, regardless of particle size within the 0.175–3 mm range. The mean motility reduction was 6.52% for PS, 5.02% for PET, 4.64% for PP, and 2.16% for PE, indicating a toxicity order of PS > PET > PP > PE.

While phenol migration from microplastics was within permissible limits in Kazakhstan, formaldehyde migration exceeded limits for all microplastic types and sizes. Formaldehyde migration increased with decreasing particle size, with the highest levels observed for PET, followed by PP, PE, and PS.

These findings suggest that both particle size and polymer structure influence microplastic toxicity. Smaller particles with larger surface areas facilitate the leaching of non-covalently bound chemicals into the aqueous phase. The discrepancy in toxicity rankings across different experiments indicates that factors beyond formaldehyde migration may contribute to the observed toxic effects and warrant further investigation.

Based on these findings, several recommendations emerge. Policymakers should implement stricter regulations on microplastic pollution in water bodies and invest in advanced water treatment technologies. Additionally, regulatory limits for water pipes and plastic containers should be reevaluated to account for the potential release of hazardous compounds like formaldehyde.

This study provides a foundational understanding of the toxicity and mutagenicity of aqueous microplastic extracts. While human exposure was not directly assessed, the results can inform predictions of human health impacts. Future research should quantify the toxic risks associated with chronic microplastic ingestion, identify safe exposure limits, and investigate the potential carcinogenicity of these particles. Moreover, future studies should expand the scope to include a wider range of microplastic types, long-term exposure assessments, and the combined effects of multiple contaminants.

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