Journal of Inonu University Health Services Vocational School
İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi

e-ISSN: 2147-7892 Volume 13, Issue 2 (2025) 529-541 doi: 10.33715/inonusaglik.1691943

Research Article

TOXIC EFFECTS OF GLYPHOSATE AS A HERBICIDE IN AQUATIC ENVIRONMENT: REPRODUCTIVE HEALTH AND SPERM QUALITY PARAMETERS

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Article Info

Received: *18 February 2025*
Accepted: *05 June 2025*

Keywords

Animal testing alternative,
Fish sperm cell,
Herbicide,
Reproduction,
Toxicity

ABSTRACT

Glyphosate is a broad-spectrum herbicide most commonly used to control weeds worldwide. The widespread use of this herbicide has raised concerns about the risk of transfer to aquatic systems and, therefore, to humans. Because terrestrial organisms and humans can be exposed to herbicides in different ways: These can be through herbicide drift into the environment, through inhalation or dermal contact, or through the use of contaminated food or water. Thus, in this study, the *in vitro* effects of different doses of glyphosate in the aquatic environment on the reproductive health of fish were studied. The sperm quality parameters of *Capoeta trutta* in the Upper Euphrates Basin were studied by the computerized sperm analysis system. According to the results, it was decreased statistically significantly ($p<0.05$) in the sperm quality parameters of Straight Linear Velocity (VSL), Curvilinear Velocity (VCL), and Angular Path Velocity (VAP) compared to the control group at all doses of glyphosate. As a result, the presence of glyphosate in the aquatic environment and its transfer from these water sources to food produced through agricultural activities may pose a serious risk to the reproductive health of both humans and other living creatures, and it may be recommended that more extensive scientific research be conducted in the relevant area.

INTRODUCTION

The overall production capacity of glyphosate has been increasing for decades, and it was 1.1 million tons/year in 2012, which far exceeded the real world demand. The Republic of China accounts for a significant portion of the overall production, and it is reported that production amounts increased by 2.6 times from 323 thousand tons/year in 2007 to 826 thousand tons/year in 2010. Statistics show that China alone can meet all the global glyphosate demand to date. While the United States dominated glyphosate production in the seventies, its share in the global annual glyphosate turnover has gradually decreased since then (Székács & Darvas, 2018).

According to 2017 data in Europe, the average active herbicide used per hectare is 0.24 kg glyphosate, while its global use is estimated at 4438.5 million dollars. In the last 20 years, glyphosate as a herbicide has increased in use, especially in North and South America, despite its known water pollutant properties, with the increase in production capacities of genetically

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How to cite this article: Özgür, M. E. (2025). Toxic Effects of Glyphosate as A Herbicide in Aquatic Environment: Reproductive Health and Sperm Quality Parameters. *İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi*, 13(2), 529-541. doi: 10.33715/inonusaglik.1691943

modified grain products (soybeans, corn, etc.). On the other hand, glyphosate, which is used excessively for weed protection in genetically modified grain products, is inevitably carried to surface waters by rain or water currents, and it is estimated that these contamination levels can exceed 5000 µg/L. As a result of glyphosate contamination of surface waters, it will likely have toxic effects on aquatic microorganisms, algae, invertebrates and vertebrates, and it will again reach the terrestrial system and human use from these waters (Coupe, Kalkhoff, Capel & Gregoire, 2012; Ogunbiyi et al., 2023; Székács & Darvas, 2018).

In chronic, developmental and reproductive toxicity studies in vivo conducted on mammals, glyphosate has been examined alone to determine toxicity limits. However, since glyphosate is never found alone in formulations, these studies are insufficient. Because it has been reported that glyphosate, which is the active ingredient of more than 750 different herbicides used worldwide, and its commercial formulations may have potential toxic effects even below the specified limit values (Mesnage, Defarge, Spiroux de Vendômois & Séralini, 2015). When glyphosate is absorbed or ingested by the body, it presents critical health concerns, such as glyphosate-based formulations being found in urine and feces and not being converted into other chemicals (Ledoux, Hettiarachchy, Yu, Howard & Lee, 2020).

When looking at the literature, it has been determined that glyphosate poses many risks to human health. For example; there is a correlation between increased exposure to glyphosate-based herbicides and the development of many diseases, including neurodegenerative diseases such as Alzheimer's and Parkinson's, autoimmune diseases, hepatic and nephrotic degenerations, infertility, thyroid, bladder, pancreas, leukemia and breast cancer (Samsel & Seneff, 2013), on the other hand, various health problems such as kidney failure and liver damage in agricultural workers (Wang, Fan, Tan, Cheng & Chen, 2011), adverse effects on the cardiovascular system (Gress, Lemoine, Séralini & Puddu, 2015), the risk of melanoma increasing by 80% (De Roos et al., 2005), children whose mothers and/or fathers are known to have been exposed to glyphosate have been shown to have an increased risk of childhood cancers such as lymphoma (Flower et al., 2004), moreover, it has been multiple myeloma in individuals exposed for long periods (>2 days/year) (Kachuri et al., 2013). While there are few epidemiological studies on the effects of glyphosate exposure on the reproductive system, it was found decreased fertility in women (Sanin, Carrasquilla, Solomon, Cole & Marshall, 2009). Premature birth infections, respiratory distress, feeding difficulties, intraventricular hemorrhage, sepsis and long-term illnesses such as cardiovascular disease, kidney disease, neurodevelopmental delays and lung impairment (Varde et al., 2023).

The studies on glyphosate in the reproductive system of terrestrial and aquatic animals as follows: in cattle embryos (Cai et al., 2020), frog (*Xenopus laevis*) oocytes (Slaby et al., 2020), larvae (Fiorino et al., 2018) and sperm (Lopes et al., 2014) of zebra fish (*Danio rerio*), the embryo of common carp (*Cyprinus carpio*) (Socha et al., 2021), sperm of coastline fish (*Jenynsia multidentata*) (Albañil Sánchez, da Costa Klosterhoff, Romano & De Martinez Gaspar Martins, 2019), sperm of grass carp (*Ctenopharyngodon idella*) (Lugowska, 2018), sperm of Nile Tilapia (*Oreochromis niloticus*) (Acar, İnanan, Zemheri Navruz & Yılmaz, 2022), sperm of Argentine silverfish (*Odontesthes bonariensis)* (Menéndez-Helman, Gárriz, del Carmen Ríos de Molina & Miranda, 2025), and sperm of trout (*Onchorhynchus mykiss*) (Akça, Kocabaş & Kutluyer, 2021).

With the increase in pollution in the aquatic environment, it is necessary to monitor the toxicity of pollutants and determine their mechanisms and effect levels in environmental risk assessment. In vitro techniques are important in the assessment of the aquatic environment, which is the main habitat of fish that reproduce with external fertilization, against pollutants (Özgür, Ulu, Sezer, Köytepe & Ateş, 2024). Again, in bony fish that reproduce with external fertilization, the movement times of sperm cells are short and tend to decrease rapidly. For example, sperm cells, whose movement starts when they meet with water, can survive for 20-25 seconds in rainbow trout (*Oncorhynchus mykiss*) and 1-2 minutes in carp (*Cyprinus carpio*) (Alavi & Cosson, 2006). For this reason, Computer-aided sperm analysis (CASA) has become very popular in recent years for fast, practical and effective analysis and has replaced traditional methods and estimation methods based on the personal opinions of sperm analysts (Kime et al., 2001; Özgür, Okumuş, & Kocamaz, 2019). However, the sperm cells of bony fish, which start moving when they come into contact with water but have a very short life span, will of course present a very disadvantageous situation if they encounter pollutants during this short life span. Considering that the main function of sperm cells is to fertilize eggs, the investigation of their survival against pollutants is one of the most important issues. In fish, despite external fertilization and reproductive activities in the aquatic environment, it is difficult to think that sperm cells exposed to toxicity from pollutants will play an active and successful role in the continuation of new generations.

The predicted environmental concentration (PEC) of glyphosate in drinking water is 0.1 µg/L according to the Environmental Quality Standard (EQS) set by the European Union Directive (EU) 2020/218470 for generic pesticides (SCHEER, 2022). Despite the very low levels of glyphosate required in drinking water by these and similar environmental authorities, it is estimated that contamination levels in natural aquatic habitats may exceed 5000 µg/L as a

result of agricultural applications (Coupe et al., 2012; Ogunbiyi et al., 2023; Székács & Darvas, 2018), explaining that the risk of aquatic glyphosate contamination is quite high. On the other hand, unfortunately, in 2022, the European Food Safety Authority (EFSA) decided to renew the approval of glyphosate for the next ten years, considering that the current data does not provide sufficient evidence to prove the mutagenic/carcinogenic effects of glyphosate. The main purpose here is to review the scientific studies that explain the potential risks to human health from glyphosate and to examine its mutagenic and carcinogenic potential and its endocrine disrupting effects on the human reproductive system (Galli et al., 2024).

Despite the existing risk possibilities and due to insufficient scientific data on its effects on the human reproductive system, it has been decided to determine the effects of sperm quality parameters with an animal test alternative as fish. Additionally, in the scientific literature, no studies have been conducted on the toxic effects of glyphosate, especially on fish living in the Upper Euphrates River Basin. So, this study was designed to investigate the effects of the nominal doses of glyphosate (0 (control), 1, 5, 10 and 25 mg/L) on the reproductive health and sperm quality of *Capoeta trutta* fish species. Therefore, the main purpose of the current study is to produce results that can give an idea about the possible consequences for both the reproductive health of fish in aquatic environments and the health of humans and communities that directly interact with water.

MATERIAL AND METHOD

Breeding Fish and Chemicals

Karabalık (*Capoeta trutta*) breeding stocks were caught from Karakaya Dam Lake in the Upper Euphrates River Basin at the beginning of May 2024, and after sperm samples were taken, the fish were released back to their habitats. Sperm samples were collected directly from 10 male fish (325±15 g weight, 27±5 cm total length, Mean±Sd.) without hormonal injection since they were in the reproductive period. Sexual maturity was confirmed by urogenital aperture and reproductive tubercles on the nose. Semen samples were collected in Eppendorf tubes by gently pressing the abdomen, and the sperm pool formed from sperm subgroups was created in a falcon tube with inactivation solution (INAS), which stops the movement of sperm cells. While taking sperm samples, care was taken to avoid contamination with bloody and fecal waste. The sperm samples were then transported with ice support to the laboratory in the Department of Fisheries Engineering of the Faculty of Agriculture at Malatya Turgut Özal University for the experiment. The sperm cell density in the sperm pool was approximately 10.2×10^9 cells/mL. The sperm samples were kept in the refrigerator at +4 ^{o}C until analyzed.

The herbicide with the commercial product name Sonround®48 SL, containing glyphosate isopropyl amine salt equivalent to 480 g/L glyphosate, was obtained from a commercial herbicide vendor. It was dissolved in distilled water and added to sperm cells in Eppendorf tubes *in vitro*.

Experimental Application

For the preparation of sperm samples and motility analysis, stock solutions were prepared to obtain inactivation solution (INAS) containing 200 mM KCl and 30 mM Tris-HCl, pH: 8.0 and activation solution (AS) containing 45 mM NaCl, 5 mM KCl and 30 mM Tris-HCl, pH: 8.0 (Özgür et al., 2020; Poupard et al., 1998). Glyphosate nominal doses were determined as 0 (control), 1, 5, 10, and 25 mg/L. 6 sub-sperm samples were taken for each dose from the sperm pool formed by taking 10 brood fish. The sub-sperm samples were diluted with INAS 100 times their amount in Eppendorf tubes, and then glyphosate prepared at different rates was added to the solution, mixed gently, and left for in vitro incubation at 4 ^{o}C for 4 hours.

Kinematic Parameters of Sperm Cells

All sperm samples were kept on ice throughout the procedure. Samples were taken after 4 hours and examined under a microscope. Sperm samples were activated with AS solution at a ratio of 1:20 and analyzed under a microscope. The final dilution ratio was 2000-fold. The dilution ratios of sperm samples were adjusted according to the 2-set procedure (Billard & Cosson, 1992). Sperm samples were examined with an Olympus BX 53 phase contrast microscope with a Sony CCD VB600B camera, 20x1.25 magnification, and video recordings were taken. The video recordings were evaluated using the BASA-Sperm Aqua module software produced by Merck Biotechnology Ltd., which is also a domestic production model of the Computer-aided Sperm Analysis System (CASA). The following parameters were examined for the kinematics of sperm cells: Sperm cell kinematics such as VSL (linear velocity, μm/s), VCL (curvilinear velocity, μm/s), VAP (angular path velocity, μm/s), LIN (Linearity values, ratio of net distance moved to total path distance, %), ALH (amplitude of lateral displacement of sperm cell head, μm) and MAD (Mean Angular Displacement, °) (Özgür et al., 2019).

Statistical Analysis

Multiple comparisons between groups of glyphosate-exposed sperm samples were performed using one-way ANOVA-Duncan test after a variance homogeneity test in each group. Normality test and descriptive analysis (Means±SE, $p<0.05$) were performed on the data.

Statistics were performed using the SPSS 15 program. Graphs were created using GraphPad Prism 5.

RESULTS AND DISCUSSION

The cytotoxicity of glyphosate at different rates on sperm cells was investigated in this study. The lower and upper limit means of the kinematic parameters of sperm cells exposed to different doses of glyphosate at the $p<0.05$ level and within the 95% confidence interval are given in Table 1. According to the data obtained at the end of the study, it was observed that the motility parameters of sperm cells, VSL (Straight Linear Velocity), VCL (Curvilinear Velocity) and VAP (Angular Path Velocity), decreased statistically significantly ($p< 0.05$) compared to the control group after glyphosate exposure. This effect began to be evident, especially in the motility speeds of sperm cells, after the 5 mg/L dose. In addition, the lowest values of these speeds were determined at the 25 mg/L glyphosate dose. Although fluctuations were observed in the LIN (Linearity Values) and ALH (amplitude of lateral displacement of the sperm cell head) values, statistically significant ($p<0.05$) differences were observed when compared to the control group. The MAD value decreased with increasing doses of glyphosate, but statistically insignificant ($p>0.05$) changes were observed compared to the control group (Table 1, Figure 1).

Table 1. Averages of Sperm Cell Kinematics of *Capoeta trutta* Exposed to Different Doses of Glyphosate, within 95% Confidence Interval and at $p<0.05$ Level.

Sperm cell kinematics	Glyphosate Doses (mg/L)	Mean±Std. Error*	95% Confidence Interval for the Mean	
			Lower Limit Value	Upper Limit Value
VSL (µm/s)	Control	68.79±5.70^{a}	54.13	83.45
	1	63.28±1.49^{ab}	59.45	67.11
	5	57.19±1.52^{b}	53.29	61.09
	10	30.48±2.10^{c}	25.10	35.87
	25	22.88±0.76^{c}	20.92	24.84
VCL (µm/s)	Control	136.82±3.63^{a}	127.49	146.15
	1	130.53±4.87^{ab}	118.00	143.06
	5	119.70±3.45^{b}	110.83	128.57
	10	90.69±5.26^{c}	77.17	104.21
	25	79.91±6.71^{c}	62.65	97.16
VAP (µm/s)	Control	71.55±4.19^{a}	60.78	82.33
	1	71.43±4.64^{a}	59.49	83.37
	5	58.31±2.89^{b}	50.87	65.74
	10	26.09±1.64^{c}	21.86	30.32
	25	20.09±2.92^{c}	12.59	27.59

LIN (%)	Control	33.46±1.19[a]	30.40	36.51
	1	34.92±1.12[ab]	32.04	37.80
	5	39.45±1.93[b]	34.49	44.40
	10	20.41±3.10[c]	12.44	28.37
	25	14.42±0.54[d]	13.02	15.81
ALH (µm)	Control	40.28±0.89[a]	38.00	42.56
	1	42.91±3.73[a]	33.32	52.49
	5	33.91±2.06[b]	28.63	39.20
	10	7.51±1.49[c]	3.68	11.34
	25	6.31±0.67[c]	4.58	8.03
MAD (º)	Control	0.04±0.01	0.03	0.06
	1	0.03±0.01	0.02	0.05
	5	0.03±0.00	0.02	.04
	10	0.03±0.00	0.03	0.04
	25	0.03±0.00	0.02	0.04

Means with different letters represent statistically significant differences at the $p<0.05$ level. VSL (linear velocity, µm/s), VCL (curvilinear velocity, µm/s), VAP (angular path velocity, µm/s), LIN (Linearity values, ratio of net distance moved to total path distance, %), ALH (amplitude of lateral displacement of sperm cell head, µm) and MAD (Mean Angular Displacement, °).

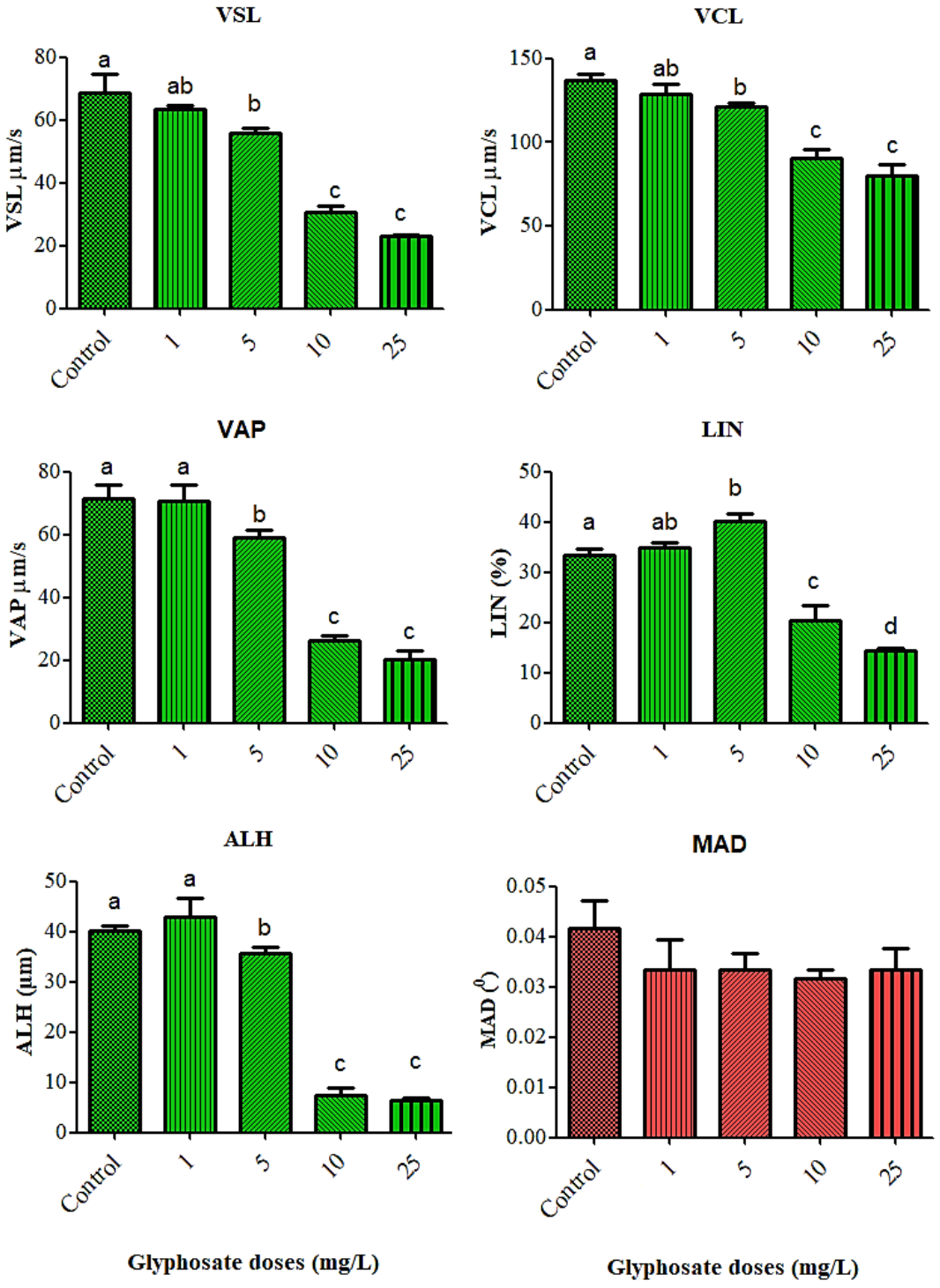

Figure 1. Sperm Cell Kinematics of *Capoeta trutta* Exposed to Different Doses of Glyphosate. Means Shown with Different Letters Represent Statistically Significant Differences at the $p<0.05$ Level. VSL (linear velocity, µm/s), VCL (curvilinear velocity, µm/s), VAP (angular path velocity, µm/s), LIN (Linearity values, ratio of net distance moved to total path distance, %), ALH (amplitude of lateral displacement of sperm cell head, µm) and MAD (Mean Angular Displacement, °).

Scientific studies demonstrating potential reproductive health effects caused by glyphosate are very limited, and additional epidemiological studies are needed to better understand these effects. However, there are a few available animal studies demonstrating

harmful reproductive health effects of glyphosate at environmentally relevant doses (Milesi, Lorenz, Durando, Rossetti & Varayoud, 2021), converted into other chemicals (Ledoux et al., 2020). According to the literature presented extensively in the introduction section, it is stated that glyphosate has negative effects on human health, especially on infertility and fertility damage (Samsel & Seneff, 2013; Varde et al., 2023), as well as carcinogenic effects (Flower et al., 2004).

Other hand, the studies in the effects of glyphosate on terrestrial and aquatic animals showed that it caused alarming results in an experiment using cattle embryos and caused embryo death, while it was reported that teratogenic effects (congenital defects in the embryo and fetus) were observed even at very low doses (0.9 mg/L) (Cai et al., 2020). Glyphosate delayed the maturation of frog (*Xenopus laevis*) oocytes (Slaby et al., 2020), and even non-lethal doses of glyphosate caused impairment in the perception of tadpoles (Moore, Chivers & Ferrari, 2015). Glyphosate at doses of 0.005, 0.05, 5, 10 and 50 mg/L impaired locomotor activity in zebrafish (*Danio rerio*) larvae exposed for 96 hours (Fiorino et al., 2018). In an acute toxicity experiment, it was reported that doses of 0.5 mg/L given to the aquatic environment for 24 or 96 hours caused damage to the liver, gills and brain of shoreline fish (*Jenynsia multidentata*) (Albañil Sánchez et al., 2019). It was reported that there was no change in sperm count in male zebrafish exposed to glyphosate at doses of 5 and 10 mg/L, but decreased sperm motility, decreased mitochondrial membrane integrity of sperm cells and DNA damage occurred (Lopes et al., 2014). It was determined that significant adverse effects were observed in carp (*Cyprinus carpio*) during embryonic development and hatching stages (Socha et al., 2021). It was reported that grass carp (*Ctenopharyngodon idella*) eggs (0.1-10 mg/L) and sperm cells (0.1-50 mg/L) were negatively affected (Lugowska, 2018). Akça et al. (2021) studied the toxicity of different glyphosate doses (2.5, 5, 10 mg/L) on trout (*Oncorhynchus mykiss*) sperm. As a result of their studies, they found that glyphosate had a harmful effect on rainbow trout spermatozoa, especially on motility percentage and duration, even at lower doses (Akça et al., 2021). Nile tilapia (*Oreochromis niloticus*) were exposed to 0, 5, 10, 20, 30 and 40 mg/L glyphosate for 14 days, and it was determined that glyphosate at 5 mg/L and above had negative effects on sperm motility parameters of the fish (Acar et al., 2022). Again, it was determined that 1, 5, 10 and 50 mg/L glyphosate in the sperm of the Argentine silverfish (*Odontesthes bonariensis*) species decreased motility at 30 seconds after activation of sperm cells (Menéndez-Helman et al., 2025), while it was reported that glyphosate reduced the motility of human sperm cells, and their quality was negatively affected (Anifandis et al., 2018). The findings of the current study were found to be supported by the results of previous studies

(Acaret et al., 2022; Akça et al. 2021; Albañil Sánchez et al., 2019; Fiorino et al., 2018; Lopes et al., 2014; Menéndez-Helman et al., 2025), and the result that glyphosate had a negative effect on the speed and kinematic characteristics of sperm cells at certain doses was similar. Again, as a result of the study, the data obtained support the suggestion that it will have a negative effect on human reproductive health (Anifandis et al., 2018).

CONCLUSION

Our study, based on the effects of glyphosate on sperm cells of the *Capoeta trutta* from the Karakaya Dam Lake in the Upper Euphrates Basin, creates perspectives on decoration for other fish in the basin. The comparative analysis of glyphosate, a herbicide, on sperm motility is a noteworthy aspect of our study. In general, our results indicate that the presence of glyphosate in water resources in natural habitats negatively affects the reproductive health of fish. However, it is thought that glyphosate exposure and its interaction with the androgen receptor agonist cause estrogenic activity in human cell lines, thus endangering human reproductive health from toxic substances in the environment (Gasnier et al., 2009; Komsky-Elbaz, Saktsier & Roth, 2018). Therefore, it is thought that humans may pose a significant risk by using glyphosate-contaminated waters for agricultural purposes and/or for any reason. It was also concluded that the data obtained as a result of the study have the potential to contribute to public health.

Acknowledgement

I would like to thank Malatya Turgut Özal University, Department of Fisheries Engineering for supporting me with laboratory facilities in my study.

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