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Examining the impact of 2-4 D amine herbicides on *Barbus luteus* Hamri fish (Heckel)

Dhurgham A A AL-Sultany[1*], Ladha Mohammed Obayes[2], Laith Taha Mohammed[1], Tabarek Najah Hadi Al-Gburi[2]
[1] Department of Biology, College of Sciences, Al-Qasim Green University, Babylon, Iraq
[2] Department of Environment, College of Environmental Science, Al-Qasim Green University, Babylon, Iraq

Abstract
The study investigated the herbicide 2-4-D-amine effects on *Barbus luteus* Hamri fish. The carp were divided into two age categories and exposed to different concentrations of the pesticide. The study also measured water properties like water temperature, pH, EC, D.O., and hardness when the fish were exposed to the pesticide.
The study reveals that fish exposed to pesticides show surface symptoms like skin and gill bleeding, neck warping, and scale loss. Compared to a control sample, these fish show irritation or confusion. The acute toxicity (LC50) of the 2-4 D amine was determined for a period of 96 hours after the food was cut off (24 hours) prior to the experiment. The first category has a greater impact on fish than the second, as demonstrated by the statistical analysis results at the probability level ($P<0.05$), which indicated an inverse link between the pesticide toxicity and the fish age at usage.
The results showed that the higher the pesticide concentration, the higher the mortality rate. The statistical analysis also showed a positive correlation between both categories and the pesticide concentration and the percentage of death (toxicity).

Keywords: 2-4 D amine, Hamri fish, herbicide, LC_{50}, and hardness

Introduction
Taking the use of herbicides enshrined increasing significantly resulting in the emergence of side effects represented in a breach occurrence in the normal balance for ecosystems and that herbicides are a potential risk of a threat to different forms of life, including humans (Edward & Thompson, 1973) [10].
The natural water is an environmental center that receives large quantities of herbicides after their use for agricultural purposes and as a result of many natural processes like carrying air and washing it from the soil by runoff water. The concentrations of herbicides in natural aquatic environments are increasing due to pollution of them (Guiho, 1995; Oemichen & Habere, 1986) [16, 25]. the result of high concentrations of herbicides is the occurrence of adverse effects on loved ones in different organisms in aquatic ecosystems, resulting in a decrease in communities of phytoplankton, zooplankton, and fish (Dewey, 1986; Shafer *et al*., 1994; and Solomon *et al*., 1996) [8, 33]. In addition, the pollution of natural water by herbicides leads to their accumulation in the organism's tissues, including fish, to high levels that pose a risk to human health when consumed (Osfor *et al*., 1998) [27].
Toxic pollution is one example of how multiple types of pollutants can accumulate as a result of agricultural effluent discharge into water bodies occurring continuously (Mason, 1991) [24]. Environmental toxicology research in non-mammalian vertebrates is becoming more and more popular as a means of assessing the impacts of harmful substances. In addition to having a negative impact on fish populations and other aquatic life, the use of herbicides indiscriminately, negligent handling, unintentional spills, and the discharge of treated effluents into natural waterways may also have long-term environmental repercussions (Akhlar, 1986).
Throughout the summer, when heavy rains might cause flooding, herbicides are frequently used to suppress water plants that could obstruct water flow (Annune *et al*., 1994) [3]. The loss of macrophytes is the direct result of herbicide addition; however, non-target creatures, such as fish, may also be impacted by the loss of food and habitat (Ervnest, 2004) [11].
Fish are one of the most widely distributed organisms in an aquatic environment, and being susceptible to environmental contamination may reflect the extent of the biological effects of environmental pollution of water (Pant *et al*., 1987) [29]. Commonly referred to as 2,4-D, 2,4-dichlorophenoxyacetic acid is a post-emergent, selective control herbicide used on residential lawns and gardens as well as in forestry and agriculture to manage wide leaf weeds. It is the most widely used herbicide in the world. 2,4, D is an herbicide that is part of the phenoxy class and can be made in an ester, amine, salt, or acid form (EPAU, 2005) [36].
The purpose of this study is to investigate how the herbicide 2-4 D amine affects the morphological and behavioral traits of the *Barbus luteus* Hamri fish.

Materials and methods
90 fish were collected from fish of different ages of *B. luteus* Hamri and divided into two groups depending on the age, as the first age group ranged between (90-180) days, while the second age group ranged between (180-360) days. Each category is divided into three replicates, as well as a sample control, and placed in plastic tanks measuring 1.5 x 0.5 x 0.5 with ten members in each basin. Plastic basins were filled with fresh tap water before putting the fish in them and left to thunder for 48 hours to ensure the depletion of chlorine from them.
The fish under laboratory conditions have been provided with air using small air pumps, such as those used in the fish Accessories, to ensure an adequate supply of dissolved oxygen for fish at a temperature ranging between (24-28) °C for not less than three days in order to adapt to the conditions of the laboratory before being used in the experiments.

Fish fed on a diet brought from the lake itself were substitute every 24 hours after eating to keep the oxygen environment in a healthy state, which was sterilized by pelvic potassium Bermnkenat. prepared concentrations of the three herbicides 2.4. D Amine (2, 4, 6) % dissolving a given weight of the pesticide in a liter of water and then took the concentrations of the three from the solution to record and by three replicates for each concentration and for the two categories both bug units, while considering the docks non-exposed control sample, and then determined the concentration of the pesticide is lethal to half the number of fish LC_{50} followers of the straight line equation.

The fish were exposed to the test substance for (96) hours. Mortalities were recorded at (24, 48, 72 and 96) hours and the concentrations which kill (50) percent of the fish (LC50) were determined where possible (Finney, 1952) [12]. Mortality was assessed at (96 hours) after the start of exposure, and dead fish were removed immediately. The (96 hours) (LC50) value was determined by probity analysis to obtain the (96 hours) (LC50) (Finney, 1971 and APHA, 2005) [5, 13].

Measured some of the physical and chemical properties of the water basin, which is the temperature of the water and measured by the mercury thermometer with a normal gradient (0–100) °C, and used the device pH meter. To measure the pH of the water basin after the calibration standard was administered intravenously, the electrical conductivity was measured by an E.C. meter of the type HANNA. She expressed the unity of the results (µs/cm) and using the DO meter factory by the company HANNA Also measured, the amount of oxygen dissolved in the water has crossed the YesUnit. Results (mg/L) were calculated based on the salinity values of electrical conductivity through the special equation (Mackereth *et al*., 1978) [22], and depended on Lin (1979). Basin water hardness was measured and expressed the unity of the results (mg/L).

Statistical analysis

The data was presented as means ±SD, with the least significant difference (LSD) used for mean separation, and the significant level was set at a probability level of P<0.05.

Results and discussion

The measurements of the physical and chemical characteristics of the water basins are displayed in Table 1.

Table 1: Physical and chemical characteristics of the measured basin experiment

Characteristics	First basin	Second basin	Third basin	Control basin
Temperature	24 ± 1	25 ± 1	25 ± 1	24 ±1
pH	7.3 ± 0.4	7.6 ± 0.3	7.4 ± 0.2	7.4 ± 0.3
E.C.	673 ± 23	680 ± 13	667 ± 13	671 ± 17
D.O.	8.7 ± 1.9	9 ± 1.2	9.2 ± 0.8	9.5 ± 0.8
Salinity	0.471 ± 0.021	0.467 ± 0.033	0.483 ±0.031	0.463 ± 0.041
Hardness	308 ± 10	310 ± 15	307 ± 15	293 ± 10

The present study results indicate the clear influence of the herbicide 2-4 D amine on the fish, where there were some morphological and behavioral signs on the fish when adding the herbicide concentrations in the three basins. The first morphological signs that appeared were getting bleeding in the gills, and when you monitor the number of strikes, the gill cover was found to be affected by increasing the concentration of the herbicide, which in turn affects breathing and thus metabolic and respiratory activity. also get clear bleeding in the skin, especially in the fish fin base, in addition to the fish's neck being twisted and its body's scales falling more frequently.

When the surroundings suddenly changed during the first exposure, the fish were aware of it and responded by stopping their swimming and staying still. After a while, they made an effort to jump and swim quickly to get out of the deadly water. Air gulping and surfacing were signs of faster opercula activity. Fish exhibited highly irregular swimming behavior in basins with elevated concentrations of 2-4 D amine, characterized by forceful body jerks. Their fins hardened, causing the muscles in their body to stretch.

Fish continuously released large volumes of mucus from their entire body, and eventually a thick coating of mucus was discovered to have accumulated in their gills and oral cavity. The color of the body became darker. Fish eventually lost consciousness and balance, began rolling, and eventually became tired and lethargic.

Finally, they tried to gulp air and drag their tail downward as they stayed upright for a few minutes with their anterior or terminal mouth up close to the water's surface. They quickly descended to the basin's bottom, and eventually the fish perished with their belly turned upward and their opercula wide open to reveal their gills.

The lethal level 50 (LC50) for recent type was calculated as 2. Sensitivities were observed across all concentrations with the LC50 being 7938 % then the LC50 for the second group was determined under 1. 5591% across all concentrations. In addition, not only does the herbicide more severely influence the initial C. carpio fish subgroup than the other subgroup, but also it is able to kill the first subgroup. Concurrently, the evidence of this inverse correlation between fish's age at employing herbicide's toxicity and the statistical analysis itself is by no means conceivable. The entity using the numerical analysis was the mere fact that there existed no significant probabilities in the two types of fish used in the experiment at a level of (P<0. 05).

In order to identify the dose or exposure concentration and the time associated with the death of 50 percent of the fish exposed to toxic materials, the LC_{50} test was used, which is expressed as parts per million (ppm) or milligrams per liter (mg/L) (Banaee, 2012) [7].

Figures (1) show the median lethal concentration LC_{50} of *B. luteus* Hamri fish was carried out in 96 hours for the first concentration of 2-4 D amin pesticide, which was valued at 0.391 ppm. while figures (2) explained the values of LC_{50} for the second concentration, which were valued at 0.426 ppm.

In addition, the values LC_{50} for the third concentration of 2-4 D amine pesticide have ranged 0.687 ppm for 96 hrs. are illustrated by the figures (3).

Comparing our current findings with the studies there was a note that the value of LC50 is not in agreement with many of the studies that have been conducted. Lack of consensus in LC50 may be attributed to variability in the experimental setup in the different studies, including fish species and their sensitivity, age, kinds of pollutant substances and toxicity sentiments (Ayat and Ahmed, 2016) [6].

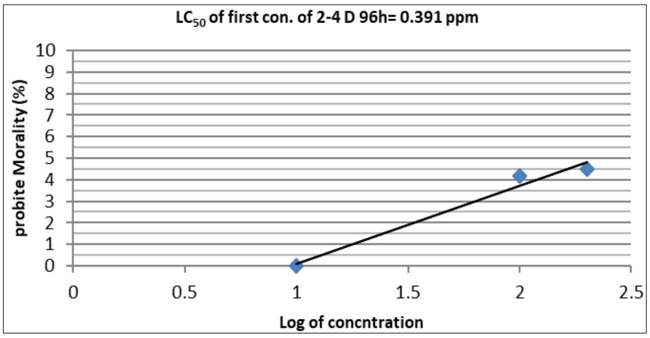

Fig 1: Median Lethal Concentration (LC_{50}) of *B. luteus* Hamri fish after (96 hr.) of exposure for first concentration of 2-4 D amine pesticide

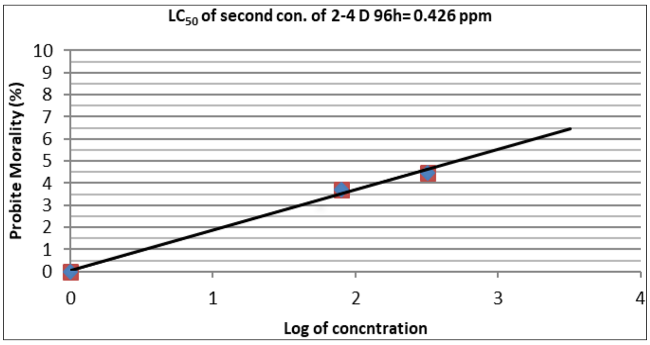

Fig 2: Median Lethal Concentration (LC_{50}) of *B. luteus* Hamri fish after (96 hr.) of exposure for second concentration of 2-4 D amine pesticide

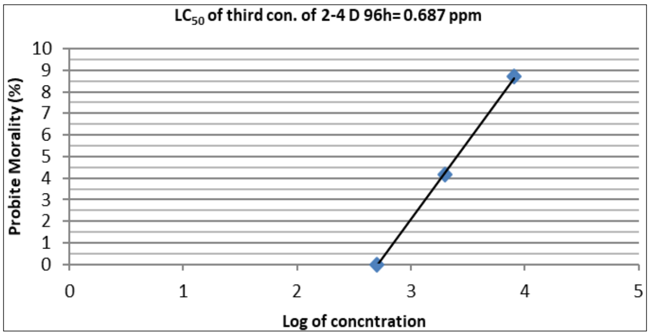

Fig 3: Median Lethal Concentration (LC_{50}) of *B. luteus* Hamri fish after (96 hr.) of exposure for third concentration of 2-4 D amine pesticide

The study findings, which showed that the herbicide concentrations and both categories had a positive correlation, indicated that there was a direct relationship between the amount of herbicides and the death rate.

The use of chemical agents such as pesticides and herbicides has increased environmental risks to fish. Some fish are used for monitoring and as sensitive indicators for environmental pollution (Seguini de Bravo *et al*., 2005) [32].

Herbicides are considered hazardous pollutants for water and cause toxicity to fish and other aquatic invertebrates. This toxicity may end up affecting a man through the food chain. Water use in agriculture and industry requires the recycling and treatment of pollutants carried by it before it reaches rivers and other sources, including drinking water and natural water (Abd-Algadir *et al*., 2011) [1].

The current research has demonstrated the distinct impact of the herbicide 2-4-D on gills. This is due to the gills usually appear to be one of the most damaged due to their direct contact with toxic substances, as they provide a very large interface between the external and internal environments of fish (Olivera Ribeiro *et al*., 2002) [26]. Some studies have shown that fish exposed to pesticides cause gill tissue damage and changes in the oxygen consumption rate. Shaffi (1979) [34] found that exposure of fish Heteropneustes fossilis to the pesticide Hetachlor caused damage to the gill tissue composition by its effect on epithelial cells and blood capillaries of the gill. The deposition of a thick layer of mublocked access led to a blockage of oxygen access to the blood. And thus failed to get the fish in need of oxygen.

Marie, *et al*., 1998 [23] As also noted in the occurrence of edema with separation of gill filaments and epithelial layer fabric in carp fish Carpinus carpio when exposed to lethal concentrations of the pesticide under the organic phosphoric Profenofos. on the other hand, Sastry *et al*. (1988) [31] found that exposure of members of the fish Channa punctatus to lethal concentrations and the fatal cause of pesticide Sevin resulted in a clear inhibition of enzyme activity Succinate dehydrogenase and Pyruvate dehydrogenase in the liver and muscles and an increase in enzyme activity Hexokinase and Lactate dehydrogenase, which indicates a rise in the rate of anaerobic metabolism in fish exposed to pesticide.

Shaikila *et al*. (1993) [35] reached a similar conclusion when exposing the fish to the pesticide Sevin. an increase in enzyme activity in acid phosphatase, whom they saw as an adaptive mutation in the tissue in order to meet its energy needs by promoting oxidative stress anaerobic, which may be due to a lack of oxygen due to the low rate of entry into the blood by gills as a result of exposure to a pesticide, and from this we can deduce the intensity of the impact of pesticides on gills.

Gomez *et al*. (1998) [15] studied the herbicide 2-4 D impact on a fish and found that the pesticide may have caused marked alteration of hemotopoietic tissue, characterized by progressive swelling and cell necrosis and activation of the phagocyte system.

There is a lot of research that shows that fish exposed to pesticides could be killed through the inhibition of enzyme activity, Acetyl Choline Esterase (AChE), when nerve and muscle nerves are received. Fulton and Key (2001) [14] have confirmed the existence of a link between the death of many species of fish and low enzyme (AChE) activity in the brains of more than 70% of these fish when exposed to pesticides.

Herrera & Catap (2001) [18] It was noted that the exposure of young fish to concentrations of 0.005 and 0.01 mg/L of the organic pesticide Azinophosethyl caused a significant decrease in enzyme activity (AChE) in skeletal muscle, as shown by the study carried out by Philips *et al*. (2002) about the effect of acute toxic pesticide Chlorpyrifos on fish on the type of Stizosadiom vitrum. The vulnerability of young fish to the pesticide caused the inhibition of enzyme activity (AChE) by up to 90%, and that these young fish aged 60 and 90 days lost their ability to survive when the ratio exceeded the inhibition of the activity of this enzyme by 71%.

After the exposure described above, the fish exhibits a variety of behavioral responses, including rapid swimming, mucus secretion, increased opercular movement, and floating on the sides. Antychowicz *et al*. (1979) [4] suggested that the fish's aberrant behavior suggests that 2-4-D amine has a harmful effect on the circulatory and central nervous systems. & Hussiein *et al*. (1996) proposed that the reduction in acetylcholinesterase activity was the cause of these behavioral alterations.

Also found in the current study were the fish in the first category, the smallest and most effective pesticide-lived fish, and the second category, older fish. This study was matched with the study by Jiraungkoorskul *et al*. (2002) [20], which studied the histopathological effects of Roundup, a glyphosate herbicide, on the Nile tilapia *Oreochromis niloticus*.

The short trial period was caused by the short half-life of the 2-4 D herbicide in water, which dissolves or degrades quickly in water and is influenced by the pH of the acidic water. Consequently, we measured the pH of the basin water used in the experiment to maintain a degree of parity and prevent the pesticide from degrading. It was found that 2,4-D responds to pH changes in water by changing both its shape and function (Que Hee & Sutherland 1981) [28]. When exposed to alkaline water, 2,4-D ionizes (becomes negatively charged), dissolves in water, and stays in the water column. The possibility exists that 2,4-D will survive longer in water with a lower pH because it will stay in a neutral molecular form and be more able to adsorb to organic particles (Wang *et al*., 1994) [37]. In muddy streams with a fine silt load, 2,4-D is most likely to adsorb suspended particles (Que Hee & Sutherland, 1981); [28] however, minimal adsorption has been seen in the field (Halter, 1980) [17].

Conclusion

Given that the herbicide 2-4-D amine is detrimental to non target species especially fish, it is liable to assume that it results in detrimental effects when overused and misused without consideration. Our study showed that the aquatic environment and the life styles of its inhabitants are heavily affected by the presence of polluted water. This is also supported by a comparison with other studies exploring the impact of contaminated water on ecosystems. The water quality was affected on many levels, including the increased pH and acidity or the lack of available water. And simultaneously it also diminishes the amount of oxygen dissolved in water. Herbicide goes first into water. The reason is the fish's close connection with outdoor environment. The fish experience elevating levels of herbicides traces in their flesh, which suffocate them by and by results in death. Upon contact with 2-4 D amine, the nervous system of water animals is badly affected which adversely affects their behavior. The sensitivity in aquatic species increased when the concentration of pesticide was greater.

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