Chapter

Impacts of Agricultural Toxicity on Non-Target Organisms in Aquatic Ecosystem

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Abstract

The present review addresses the impacts of pesticides used in crops on non-target organisms in aquatic ecosystems. In recent decades, these ecosystems have received large amounts of these compounds, which are released by urban communities, rural and industrial properties. Pesticides reach the aquatic environment through different routes (leaching, irrigation, drainage, and surface runoff) and can easily reach non-target organisms, such as fish, mollusks, as well as other benthic organisms. Usually, these animals tend to undergo bioaccumulation. Exposure to these pesticides can cause numerous physiological changes by direct influence on certain cellular structures, such as on the lysosomal membrane, which can be degraded. Also, they can even react with nucleic acids resulting in several genetic injuries, thus causing adverse reactions to the body. There is a need for more incentives for the adoption of sustainable agroecological practices, as well as a ban on active ingredients harmful to the environment, in addition to strict inspection by competent environmental agencies.

Keywords: environmental impact, pesticides, aquatic organisms, non-target organisms, aquatic ecosystems

1. Introduction

In the last decades, with the machinery modernization and the consolidation of the sector of modern inputs, agriculture has been growing at a fast pace, with pesticides being one of the main instruments that drive the agricultural sector in productivity gains [1]. However, the indiscriminate use of these substances has easily reached non-target organisms and their effects on the environment are varied, ranging from the reduction in the availability and quality of water to the compromise of air and food quality, harming human health. Also, it can directly affect cellular structures of aquatic or terrestrial organisms resulting in damage to biodiversity [2].

In the early 1960s, society began to worry about the adverse effects and potential risk that these pesticides posed to human health and the environment. In several countries, production, marketing, and use of many of these compounds, in particular those considered persistent organic pollutants (POPs), such as organochlorines, were banned [3]. With the ban on most organochlorine compounds (less toxic, but with greater bioaccumulation in the environment), after the Second World War, carbamate and organophosphate pesticides had their use intensified. Also, it became the most used pesticides worldwide, being widely used in developing countries with a predominantly agricultural economy [4].

These toxic substances have the potential to cause various biochemical and genetic injuries to non-target organisms. Carbamates and organophosphates, for example, are potent inhibitors of the acetylcholinesterase enzyme, which damages the nervous system of an exposed organism [5, 6]. This enzyme acts in the hydrolysis of the acetylcholine neurotransmitter in cholinergic synapses. Its inhibition can lead the individual to death due to cholinergic hyperstimulation. Pesticides are also known for their mutagenic and carcinogenic effects. They react with nucleic acids causing adverse reactions in the body. Thus, monitoring and controlling the presence of these substances in the environment are necessary, since these compounds have become a human health and environmental problems [7].

2. Pesticides in aquatic environments

Pesticides or agrochemicals are defined as:

> *"Products and agents of physical, chemical or biological processes, intended for use in the sectors of production, in the storage and processing of agricultural products, in pastures, in the protection of forests, native or implanted, and of other ecosystems and also of urban environments, whose purpose is to change the composition of flora or fauna, in order to preserve them from the adverse action of living beings considered harmful" [8].*

According to the harmful species that intend to eliminate, these compounds are classified as insecticides, fungicides, herbicides, acaricides, rodenticides, molluscicides, among others. Herbicides represent 48% of the total pesticides, which is followed by insecticides (25%) and fungicides (22%) [9]. Depending on the chemical class, they can be grouped into pyrethroids, organochlorines, organophosphates, carbamates, benzoylureas, neonicotinoids, among others [10].

Pesticides arrive in the environment carried by runoff and leaching of rainwater, irrigation, and drainage or by spraying, as shown in **Figure 1**. Among these processes, runoff and leaching can contaminate reservoirs, lakes, and rivers. Also, they expose aquatic organisms at levels of pesticides that can be toxic to many species. Once present in the aquatic environment, these compounds can penetrate the organisms orally - through the ingestion of contaminated food, respiratory - through the gills, and dermal - through the surface of the body. In most cases, these organisms tend to suffer bioaccumulation [1, 13]. Pesticide exposure can cause numerous physiological changes by direct influence on certain cellular structures, for example, on the lysosomal membrane, which can be degraded or can react with nucleic acids, resulting in several genetic injuries that cause adverse reactions in the body [2].

Currently, there is a growing concern about the exacerbated use of pesticides since, in recent decades, aquatic ecosystems have received alarmingly large amounts of these compounds, which are released by urban communities, rural properties, and industries. Thus, society started to worry about the adverse effects of these substances and their potential risk [14]. According to Silva et al. [4], carbamates and organophosphates are the most used pesticides worldwide. They together account for more than 50% of what is marketed.

Organophosphate pesticides (OPs) comprise a large number of substances classified chemically as esters, amides, or derivatives of pentavalent phosphoric acids. Carbamates (CBs) are esters, or N-substituted derivatives of carbamic acid (carbamic acid monoamide) (**Figure 2**). Both have low water solubility and are, in general, easily hydrolyzable in alkaline environments [10, 15, 16]. In general, OPs need biotransformation to become toxicologically active, unlike CBs that are already bioactive.

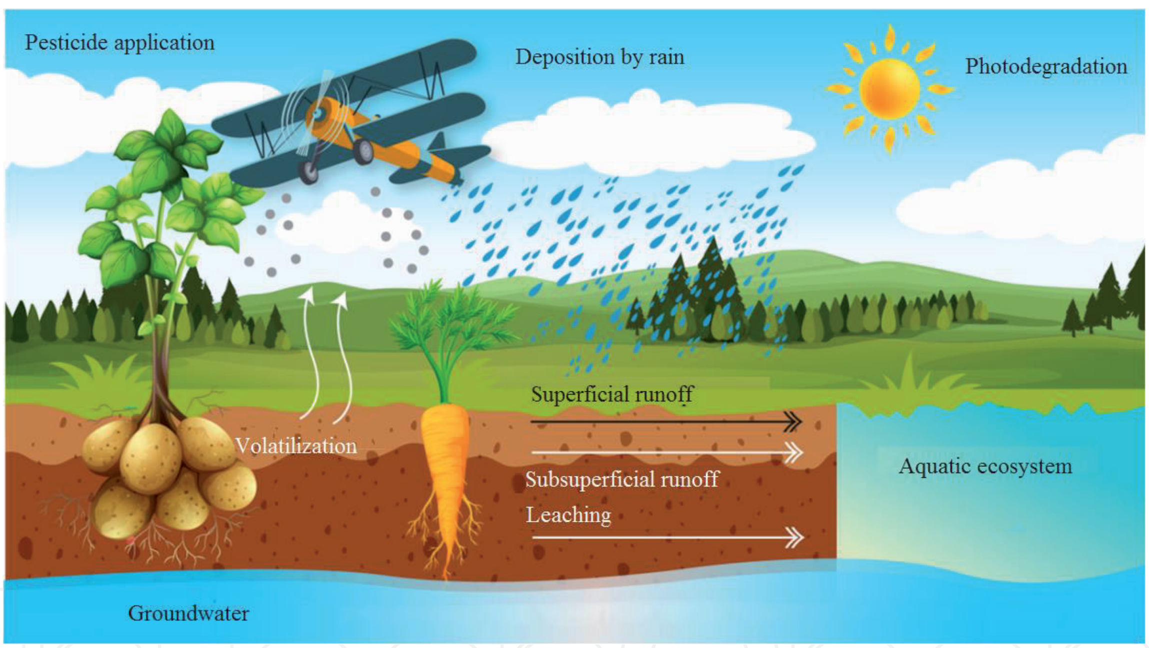

Figure 1.
Pesticide paths to the aquatic environments. Source: [11, 12].

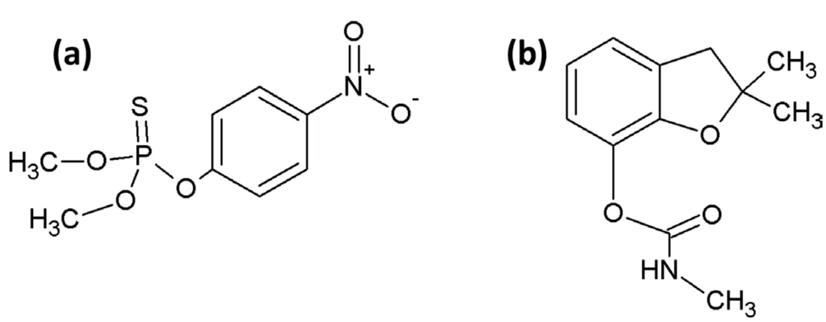

Figure 2.
Organophosphate pesticides: (a) methyl-paration (triesters of phosphoric acid), (b) carbamate carbofuran (esters derived from carbamic acid).

Carbamates and organophosphates affect the nervous system of organisms. They inhibit the activity of the enzyme acetylcholinesterase (AChE), as demonstrated by Wang et al. [17]. In their study, AChE inhibition in carp (*Cyprinus carpio*) exposed to various concentrations of organophosphates, malathion, and triazophos, as well as carbamates fenobucarb and carbosulfan, was evaluated. In equitoxic mixtures, the authors noted that AChE activity was inhibited by the combination of triazophos and malathion, as well as triazophos and carbosulfan, with synergism occurring. The effects of organophosphates on the behavior and activity of the AChE of zebrafish larvae have also been studied, through exposure to chlorpyrifos and malathion, and changes in swimming speed (hypoactivity and hyperactivity), rest and tigmotatism have been found [18].

Recently, benzoylurea, a class of pesticide that in the past was not considered an acetylcholinesterase inhibitor, since its main mode of action is the inhibition of chitin biosynthesis in insects (which interrupts the incorporation of N-acetylglycosamine monomers), demonstrated anticholinesterase potential [19]. In 2011, this class of pesticides represented 3.6% of the world's pesticide market. Since then, its commercial importance has grown over the years [20].

3. Effect of pesticides on enzymes of non-target organisms in the aquatic environment

The intensive use of pesticides in agricultural cultivation has been one of the main problems responsible for the contamination of aquatic ecosystems. It is due to both the deposition and consequent accumulation of these contaminants in the environment and the sensitivity of the organisms. Currently, there is an increasing number of studies in which fish, for example, are used as indicators of pesticides in the aquatic ecosystem, since these substances, even in low concentrations, can affect their physiology and survival capacity [17, 21, 22].

These organisms are sources of biologically active molecules. When their functioning is altered, compromise the organism's physiological functions, which culminates in genetic, biochemical, morphological, ecological, or behavioral changes [23]. These biomolecules are considered as biomarkers, and their measurement has been used in biomonitoring programs to detect exposure to toxic substances in the aquatic environment [24]. This early detection allows identifying the presence of the contaminant, even before it causes significant changes in the health of the exposed individuals.

Among exposure biomarkers, recent studies showed great interest in enzyme biomarkers as an alternative for monitoring impacted aquatic environments due to their high specificity and speed in responding to changes from target substances [4, 6, 17, 21, 25, 26]. The use of enzymes as biomarkers is based on inhibitory or inductive interference caused by contaminants in their catalytic activity. Most of these toxic compounds have a high affinity for electron pairs found in the amino acids that form the enzymes, such as the sulfhydryl - SH groups and other functional groups from the catalytic site [5, 27]. Among the main enzymes used extensively for this purpose, cholinesterase enzymes stand out (ChEs; EC 3.1.1.x).

3.1 Influence of pesticides on cholinesterase enzyme activity

Two distinct cholinesterases are found in vertebrate and invertebrate aquatic organisms, acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). AChE is a hydrolase that predominates mainly in erythrocytes, neurons, ganglia of the autonomic nervous system, and terminal motor plates. Its main function is to promote the hydrolysis of the neurotransmitter acetylcholine. It releases acetate and choline in the cholinergic synapses. Due to its key function in the control of synaptic transmission, this enzyme becomes one of the most vulnerable molecular targets to the action of neurotoxic agents. For this reason,

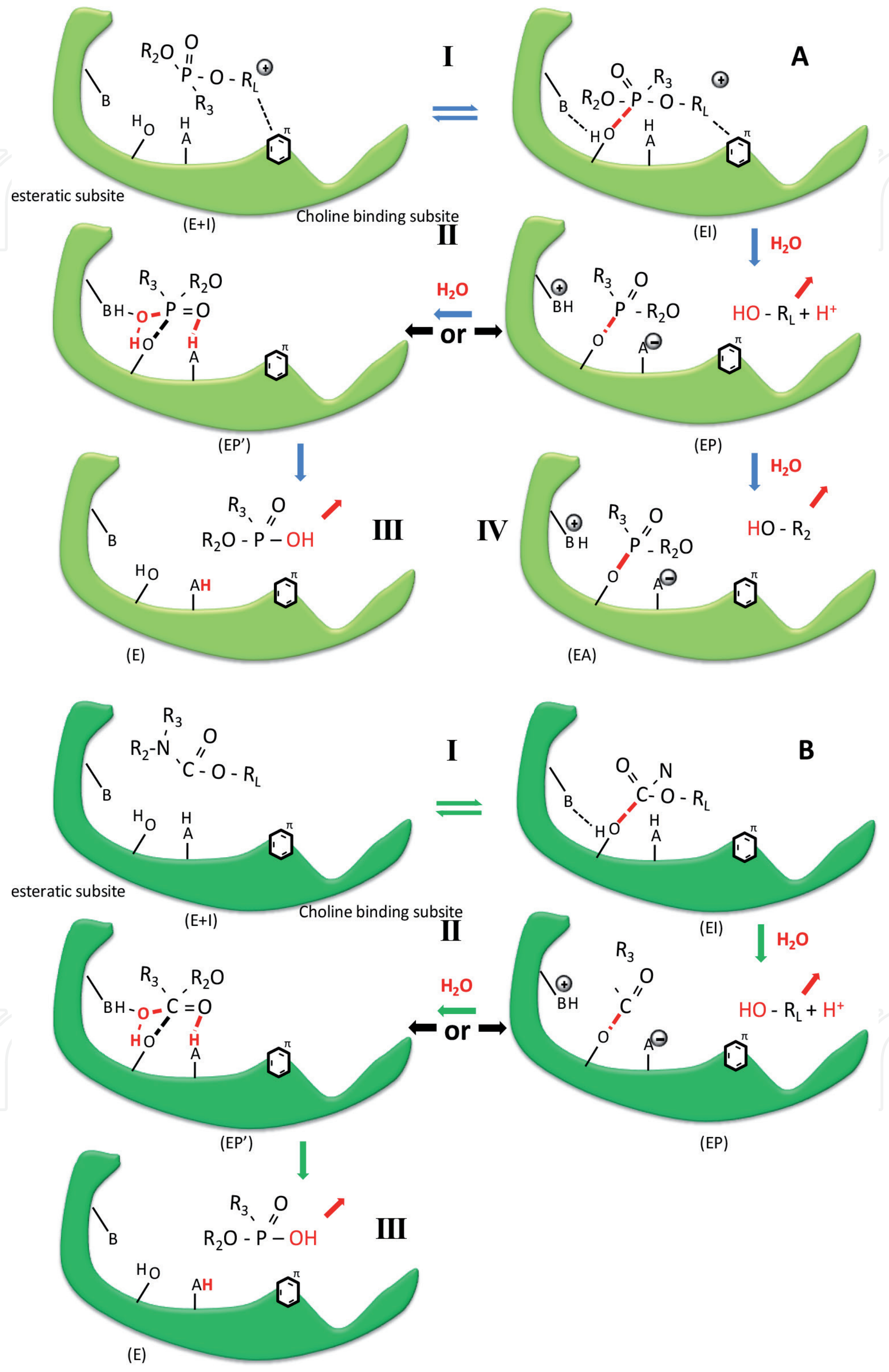

Figure 3.
Steps of inhibition by organophosphorus (A) and carbamates (B): I – Approaching of the organophosphorus (OP) or carbamate (CB) pesticide into the bottom of the catalytic cavity attracted by the choline binding sub-site (for OP only) and transition state in the interaction between enzyme and the pesticide. In particular, the bonds involved; II - Scheme representing the two occurrence possibilities during the existence of the enzyme-OP complex: spontaneous reactivation (left) or aging (right – only OP); III – Free enzyme; IV - Before undergoing aging (only OP), R2 was attracting electrons from the phosphorus atom. After the removal of R2, these electrons are shared with "O"-Serine, strengthening the binding, which cannot be hydrolyzed.

it has been widely studied in aquatic organisms and proposed for use in monitoring programs, given its sensitivity [5, 6]. On the other hand, BChE predominates in plasma, liver, neuroglia, pancreas, and digestive tract walls. It has not fully clarified its function, and the absence of its activity has been reported in the brains of several fish species [4, 28].

These enzymes are widely used in biomonitoring of aquatic ecosystems. They are used as biomarkers of the presence of two specific classes of pesticides: carbamates and organophosphates, which generally have low environmental persistence, especially when compared to organochlorines, but with greater toxicity. These substances act by inhibiting enzymatic activity. It interacts with the steratic site by phosphorylation (organophosphates) or carbamoylation (carbamates) (**Figure 3**) [12, 29].

Inhibition, once initiated, tends to generate acute or chronic intoxication. Depending on the degree of exposure to the toxic substance, the individual may die, due to over-stimulation of his nervous system, since with AChE inhibition, acetylcholine accumulates in neuromuscular junctions and cholinergic synapses [22, 30]. The signs and symptoms of carbamate poisoning are similar to those of organophosphates. They differ only in the duration and intensity of toxicity. The moderate effects of carbamates compared to organophosphates are due to the fact that they reversibly inhibit acetylcholinesterase (hydrolysis with enzyme regeneration) and are rapidly metabolized *in vivo* [31].

The anticholinesterase action of these pesticides, simultaneously, causes AChE inhibition of central and peripheral nervous tissue. Also, they inhibit erythrocyte AChE and plasma BChE [29]. According to the data from the Food and Agriculture Organization (FAO, 2007) [32], inhibition of cholinesterase activity from 20% characterizes the action of anticholinesterase agents. After 50% inhibition, clinical signs are visualized, and after 90% inhibition, the organism dies.

The *in vitro* study of acetylcholinesterase activity in various fish species, such as arapaima (*Arapaima gigas*), peacock bass (*Cichla ocellaris*), tambaqui (*Colossoma macropomum*), zebrafish (*Danio rerio*), jaguar cichlid (*Parachromis managuensis*), streaked prochilod (*Prochilodus lineatus*), cobia (*Rachycentron canadum*), and tilapia (*Oreochromis niloticus*), have been proposed to be used in the detection of harmful physiological effects of pesticides to these aquatic organisms [4, 6, 33–35]. In addition to these, we can mention the works of GHAZALA et al. [26], who tested the effect of three sublethal concentrations of the profenofos and carbofuran pesticides on the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the brain, gills, muscle, kidney, liver, and blood of the species *Labeo rohita* (Indian carp). These authors found that exposure to both pesticides affected the functions of these organs, including metabolism and neurotransmission. Araújo et al. [6] also reported *in vitro* inhibition of acetylcholinesterase by carbamate and organophosphate pesticides in the brain of the Jaguar cichlid, showing high degree of toxicity.

These studies have confirmed fish as a practical and economically viable source of acetylcholinesterase, which is capable of making water resource biomonitoring procedures routine.

4. Genotoxic effects of pesticides

Pesticides, in general, are known to have genotoxic, mutagenic, and carcinogenic action, since they interact chemically with the genetic material, promoting changes in the DNA molecule. These alterations in the organisms' DNA can cause serious consequences, since, at the individual level, they damage cells and organs and can even affect their reproductive function [36]. Among the most used methodologies for assessing DNA damage in aquatic organisms, the micronucleus (MN) test stands out, which allows the observation of macrolesions in the genome quickly, simply, and minimally invasive [37–39]. This test consists of a blood smear on a slide (**Figure 4A**) and is commonly applied to fish erythrocytes, oysters hemocytes, and crabs as an alternative in the detection of genotoxic agents, such as pesticides, in environmental biomonitoring programs [36, 40–42].

Currently, it is one of the most used cytogenetic tests in the field of toxicological genetics since it is a sensitive test for detecting structural either-or numerical chromosomal changes [43, 44]. In addition to the micronucleus test, nuclear morphological changes (NMC) can also be analyzed. Several studies describe the presence of these changes in fish cells as a result of exposure to genotoxic substances [7, 44, 45].

In addition to these tests, the Comet Assay (single cell gel electrophoresis assay) is also one of the most used in the evaluation of genomic damage caused by pesticides. It presents high sensitivity in detecting pre-mutagenic lesions in individual cells. It is a technique capable of detecting microlesions in DNA, which are genomic lesions that can be repaired [46]. In this technique, cells that have damages in their DNA, form different fragments which tend to migrate at different speeds during the electrophoretic run, forming a comet under fluorescence microscopy (**Figure 4B**).

Among the studies that demonstrate the action of pesticides in aquatic organisms, we can mention the study by Silva et al. [7] that evaluated the genotoxic potential of the herbicide trifluralin (one of the herbicides most used in weed control) on *Colossoma macropomum* (tambaqui). The mutagenic and genotoxic effects of different concentrations of trifluralin (0.25, 0.5, 0.75, 1.0 mg L^{-1}) in peripheral erythrocytes of *C. macropomum*, were investigated using the micronucleus test (MN), assay comet, and apoptosis. After an exposure period of 96 h, the results showed a significant rate of micronuclei and nuclear abnormalities in erythrocytes from *C. macropomum* exposed to 0.5, 0.75, 1.0 mg L^{-1} of trifluralin compared to the group control, thus confirming the genotoxicity of the herbicide trifluralin in the investigated species.

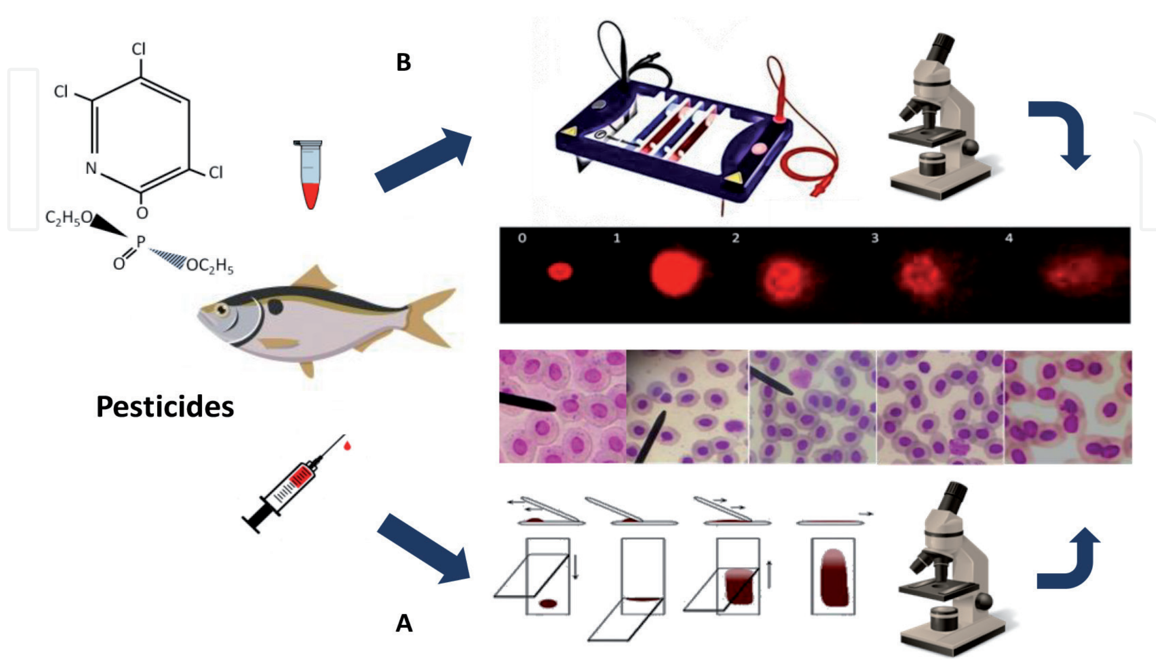

Figure 4.
Micronucleus test (A) and Comet assay (B) to evaluate DNA da mages in aquatic organisms.

5. Conclusion

In the search for high productivity, the use of pesticides has been intensified in agricultural crops. Also, the indiscriminate use of these substances has reached non-target organisms, causing deleterious effects on biodiversity, especially in the aquatic ecosystem.

To mitigate these impacts, several methodologies have been used to detect exposure to these toxic substances in aquatic environments. Among them, the methodologies that aim to evaluate the exposed organism at the biochemical and genetic level, as described in this review, show efficiency. It allows the early identification of the presence of the contaminant even before it causes significant changes in the health of the exposed individual, as well as before higher levels of biological organization are reached. It is worth mentioning that the pesticides present in aquatic ecosystems can accumulate in high concentrations in the organisms throughout the trophic level reaching the human being.

Monitoring and controlling the presence of these substances in the environment is necessary since these compounds have become a human and environmental health problem. Allied to this, there is a need for more incentives for the adoption of sustainable agroecological practices, as well as the prohibition of harmful active ingredients to the environment, added to the strict inspection by competent environmental agencies.

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