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Pesticide residues detected in *Colossoma macropomum* by the modified QuEChERS and GC-MS/MS methods

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ABSTRACT. This study aimed to detect pesticides in the muscle tissue of farmed tambaqui (*Colossoma macropomum*), in relation to good management practices (GMP) and prophylaxis and biosecurity measures. There were 54 fish farms randomly selected from the 138 found in the Microregion of Zona da Mata, RO - Brazil, for visits and collection of epidemiological data. There were extracted 24 muscle fragments were extracted from dorsolateral portion of the tail of five specimens tambaqui per fish farming. The methods of detection and quantification of pesticide residues were modified QuEChERS and GC-MS/MS. Most fish farms are small businesses and their production areas are smaller than a rural module. These ventures are an income alternative for rural producers, this information is confirmed in percentage of 88.89% (48/54) of the rural properties visited have livestock and agriculture as their main productive activity. Water monitoring was carried out in 70.37% of fish farms. However, with frequency of two water analyzes per year. Preventive and prophylactic measures taken were performed in 30% (17/54). In addition, 7% (4/54) of fish farmers reported using sodium chloride and/or using formalin as a secondary preventive measure. It is also important to mention that there was a report of administration of potassium permanganate in a fish farm, 2% (1/54). There were 12.49% (3/54) positive for at least one pesticide. The chemical compounds found Azosxystrobin (<LOQ), Epoxiconazole (<LOQ) and Chlorpyrifos (<LOQ). The presence of Azosxystrobin, Epoxiconazol and Chlorpyrifos in tambaqui muscle tissue samples was detected above acceptable limits by the PNCRC.

Keywords: Environmental toxicology; fish farming; fish technology; chromatographic analysis; technology for aquaculture.

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Introduction

The indiscriminate use of insecticides, fungicides, herbicides and veterinary drugs are sources of pollution harmful to the aquatic environment. This fact has contributed to the ecological impact on rivers, lakes and dams (Rodrigues, Lopes, and Pardal, 2013; Mesak et al., 2019). The poor use of these compounds has been causing contamination of target and non-target organisms in the environment (Mello and Silveira, 2012; Pignati, Souza, Mendes, Lima, and Pezzuti, 2018). The pesticides, when applied to plant and to the soil, tend to move mainly to surface and groundwater, and reach the bodies of water, directly, or through rainwater and irrigation (Mahboob, Al-Ghanim, Al-Misned and Ahmed, 2014). As much as, indirect contamination can occur through the volatilization of compounds applied to crops and by the formation of dust from contaminated soil (Rodrigues, Souza, Rocha, Costa, and Mendes, 2017). Therefore, fish are considered bioindicators of environment quality. Due to exposure to genotoxic compounds, fish are sentinels that react to changes at various structural levels, from behavioral factors, metabolic, cellular, physiological, histological disorders to variations in behavior patterns, which can cause changes in the dynamic characteristics of populations (Lima, Morais, Andrade, Mattos, and Moron, 2018).

The species examined was tambaqui *Colossoma macropomum* Cuvier, 1818 (Characiformes: Serrasalmidae). Tambaqui is a fish native to Amazon basin, grown throughout the Brazilian territory, and has achieved a production of 29.3% of fish production in the country (*Instituto Brasileiro de Geografia e Estatística* [IBGE], 2018). The Rondônia state is the largest producer of natives fish in Brazil, accounting for approximately 47.5% of the production of native species (Peixe BR, 2021). However, the health of *C. macropomum* can be affected by changes caused by different chemical compounds, and some, with bioaccumulative potential (Souza, Campeche, Campos, Figueiredo, and Melo, 2014; Valenti, Barris, Moraes-Valenti, Bueno and Cavalli, 2021). In addition to impacts on the environment, there are several cases of intoxications and other human health problems caused by the consumption of food contaminated by pesticides (Lopes and Albuquerque, 2018).

Assuming the need for technologies to optimize the quality of products from animal production, with a focus on fish farming. The economic exploitation of fish farming requires basic knowledge of the main factors that are directly or indirectly linked to environmental toxicity. In view of the lethal or sublethal effects of pesticides, and impacts on aquatic ecosystems, as well as the ability to harm organisms of different trophic levels. Therefore, it is necessary to carry out a toxicological study of several chemical substances, since several pesticides are periodically launched on the market, most of them without any adequate evaluation from the point of view of their interaction in ecosystems (Bernardi, Moraes, Varoli and Osti, 2008).

Given the assumptions, this study aimed to detect and quantify pesticides in the muscle tissue tambaqui farmed in small-scale fish farming in the Western Amazon region, using the QuEChERS modified method and Liquid Chromatography Coupled to Mass Spectrometry in Series (GC-MS/MS) and relation with good management practices (GMPs) and prophylaxis and biosafety measures adopted in the properties' crops.

Material and methods

Bioethical approval and general data

The research activities and protocols were approved by Ethics Committee on the Use of Animals (CEUA), Universidade Federal de Rondônia (UNIR), under protocol number 21/2018. The study area was the Microregion of Zona da Mata, Rondônia state, located in the Western Amazon, Brazil. Currently, agriculture, livestock, food industry and plant extraction are the foundations of the local economy (IBGE, 2018). This region occupies a geographical area of 24,525,997 km² and 250,643 inhabitants (IBGE, 2017). The identification of properties with fish farming activities in the region was obtained from database of the Agrosilvopastoril Sanitary Defense Agency of the Rondônia State (IDARON). According to data collected at IDARON, there are 138 fish farms with an average production of three tons of fish per year.

Data collect

A total of 54 fish farms were randomly selected from the Microregion of Zona da Mata, Rondônia state, Brazil. The fish farmers answered the semi-structured interviews and were obtained epidemiological data on good practices in Aquaculture, prophylaxis, use of veterinary products and use of pesticides in the sanitary management of fishes on farm tanks.

During the conduct of the study, from October to December 2019, the fish farms were again visited to collect samples of muscle fragments from dorsolateral portion of the tail of five specimens tambaqui per fish farm. It should be noted that the muscle tissue samples were taken only from fish with ideal weight for sale, 2.0 to 2.50 kg according to Cavali et al. (2022) and Dantas Filho et al. (2022). Thus, a total of 29 fish farms were visited to remove muscle tissue (Table 1). It is important to emphasize that, to capture and contain the fish, nets and strainer were used, which made it possible to apprehend the animals with as little stress as possible. The fish were slaughtered by hypothermia (bath of approximately 0° C, mixture of water and ice in a 1:1 ratio), were Ministério da Agricultura Pecuária e Abastecimento [MAPA] (2019) guidelines followed.

An anatomical part 8 cm long by 2 cm wide was sectioned, which corresponded to 10g of the heart-ventral part of the dorsal fin to the blood-dorsal pelvic fin. The were identified with the data referring to tanks of the fish farms and stored in freezer and kept at -20º C. For sending to the Laboratório de Análises de Resíduos de Pesticidas (LARP) at the Universidade Federal de Santa Maria (UFSM), in Santa Maria city, RS, Brazil. The samples were packed in transparent plastic bags inside an isothermal box on an approximately 10 cm layer of ice and the addition of the bronopol preservative. Then, another layer of crushed ice (20 cm layer of ice) was provided in sufficient proportion to maintain refrigeration (between 0 and 5º C) and were kept in this condition until the moment of the analysis.

Table 1. Data obtained from collections of samples muscular tissue tambaqui (*Colossoma macropomum*) farmed in the Microregion of Zona da Mata, Rondônia state, Western Amazon, Brazil.

Municipalities	Identifications	Collection dates	Samples
Alta Floresta D'Oeste	Prop 01	10/01/2018	1
	Prop 05	10/08/2018	1
	Prop 07	10/22/2018	1
	Prop 12	11/12/2018	1
	Prop 08	10/29/2018	1
	Prop 09	10/29/2018	1
	Prop 23	12/03/2018	1
	Prop 24	12/09/2018	1
Alto Alegre dos Parecis	Prop 04	10/08/2018	1
	Prop 10	11/105/2018	1
	Prop 16	11/12/2018	1
	Prop 19	11/19/2018	1
	Prop 21	11/26/2018	1
Santa Luzia D'Oeste	Prop 06	10/22/2018	1
Novo Horizonte do Oeste	Prop 18	11/19/2018	1
	Prop 22	12/03/2018	1
Rolim de Moura	Prop 02	20/01/2018	1
	Prop 03	10/01/2018	1
	Prop 11	11/05/2018	1
	Prop 13	11/12/2018	1
	Prop 14	11/12/2018	1
	Prop 15	11/12/2018	1
	Prop 17	11/12/2018	1
	Prop 20	11/19/2018	1
Total	24	-	24

QuEChERS modified method and GC-MS/MS

The determination of pesticide residues in tambaqui muscle tissue was carried out based on studies described by Oliveira et al. (2019), Munaretto, May, Saibt and Zanella (2016). The extraction of tambaqui samples was performed using the QuEChERS modified method using acetonitrile acidified with formic acid as the extraction solvent followed by addition of sodium chloride and sodium acetate partition salts, in addition to magnesium sulfate. The cleaning step of the extracts was carried out with PSA and C_{18} sorbent materials.

For the determination of pesticides, gas chromatography coupled to mass spectrometry in series (GC-MS/MS) was used with an Intuvo 9000 GC and a 7010B triple-quadrupole MS, both from Agilent Technologies (USA). For quantification, an analytical curve prepared in the blank matrix and acquisition mode of ions by monitoring selected reactions (Selected Reaction Monitoring SRM) were used (Munaretto et al., 2016).

Database organization

The data obtained were stored and organized in Epi info™ software, version 3.5.3 - 2011 (OS: MS-Windows, Programming language C Sharp) and the information on detected pesticides was interpreted according to Matos et al. (2019) and according Normative Instruction No. 20, of July 26, 2018, for the LOD (mg kg^{-1}), <LOQ (mg kg^{-1}) and LRA (mg kg^{-1}).

Results

Mostly of fish farms in the Microregion of Zona da Mata, RO, are small enterprises and their production areas are smaller than a rural module. These ventures are an income alternative for rural producers, this information is confirmed in the percentage of 88.89% (48/54) of the rural properties visited have cattle raising and agriculture as their main productive activity. It is worth noting that, in this study, the species tambaqui is farmed in 100% of the fish farms visited, although other fish are commonly produced, as secondary species, for example, *Pseudoplatystoma corruscans* (surubim), *Arapaima gigas* (pirarucu) and others. Furthermore, fish farms adopt the intensive cultivation system, and the main production strategy is in excavated tanks, with the use of one to three tanks.

Therefore, was 16.67% (9/54) of the fish farms managed the following subdivisions of the production phases, it was a tank for growing, fattening and finishing the fish. And, in 81.48% (44/54) of the fish farms used at least two tanks for the same stages of production mentioned above. However, only one fish farm used three tanks, one for each stage of cultivation. Furthermore, water monitoring is carried out in 70.37% of fish farms, with a frequency of two water analyzes per year, as reported by fish farmers.

Concerning as preventive and prophylactic measures taken, were conducted in 30% (17/54) of the fish farms visited, the post-draining liming of the tanks was the most frequent disinfection strategy 89% (48/54), followed by emptying 63% (34/54) and quarantine 57% (31/54). Concerning as use of prophylactic baths, was 46% (25/54) of fish farms performed this preventive procedure. In addition, was 7% (4/54) of fish farmers reported the use of Sodium chloride and/or the use of formalin as a secondary preventive measure. It is also important to mention that there was a report of potassium permanganate administration in one fish farm, 2% (1/54).

Concerning to use veterinary antibiotics in the treatment of illnesses, was 55% (30/54) of fish farmers reported adding veterinary antibiotics to pond water and 15% (8/54) administer these veterinary drugs mixed with feed. Of the 30/54 drugs, the use of antibiotics was the most frequent among drugs 50% (15/30), antiparasitic 17% (5/30), fungicides 7% (2/30) and medicinal plants 27% (8 /30). It is noteworthy that the medicinal plants reported by the producers used empirically were not identified. In addition, it was also verified the use of pesticides to control aquatic plants in tanks in 54% (29/54) of fish farms. Faced with this problem, the chemical most used by fish farmers to control aquatic plants was glyphosate (Roundup®). In addition to this chemical, another agricultural defensive was also used by fish farmers, it was Flumyzin 500® which has flumioxazine as active ingredient.

Parasitized fish were reported in 7% (4/54) fish farms, all located in the Espigão D'Oeste municipality, in the Microregion of Zona da Mata, RO. It is worth emphasizing that *Perulernaea gamitanae* was reported in all fish farms visited in this municipality, being present in tambaqui in adulthood, and was more frequent during the period from August to November 2019. As for this problem, the treatments used by fish farmers to combat this parasitosis were baths based on sodium chloride and Neguvon® (Trichlorfon). As for good management practices (GMPs), in the Microregion of Zona da Mata, RO, biosafety measures were not observed in fish farms, such as disinfection of facilities and equipment, access control for people and vehicles, and vector and pest control in the aquaculture establishments visited, as established by the Program National Aquatic Animal Health in Farming (USDA, 2020).

After collecting data related to GMPs, samples tambaqui muscle fragments raised on the fish farm were collected. However, in 41% (22/54) it was not possible to harvest muscle tissue from the fish because there were no fish with market, weight above 2 kg. In addition, in 5% (3/54) of the fish farms, not even the sanitary emptiness procedure of the tanks was carried out, which had high infestation by Acanthocephalus.

According to the results obtained in the determination of pesticide residues by analysis of the modified QuEChERS method, 83 chemical compounds were analyzed, and 78 pesticides and five veterinary antibiotics were analyzed, among the following chemical groups: organophosphates, avermectin, pyrethroids, triazines, among others (Table 2).

It was verified that 12.49% (3/24) of the samples were positive for at least one pesticide. The chemical compounds found were Azosxystrobin (fungicide) (<LOQ), from municipality of Alto Alegre dos Parecis; Epoxiconazol (ectoparasitic and fungicide) (<LOQ), detected in a fish farm from municipality of Rolim de Moura; and Chlorpyrifos (carpathicide, acaricide, insecticide and anticide) (<LOQ), from a fish farm in the municipality of Alta Floresta D'Oeste, all in the Microregion of Zona da Mata, RO, in the Western Amazon region.

Table 2. Detection of pesticide residues found above the limits recommended by legislation, in the muscular tissue of tambaqui (*Colossoma macropomum*) farmed in the Microregion of Zona da Mata, Rondônia state, Western Amazon, Brazil.

Pesticide	Confirmed samples	LOD* ($mg\ kg^{-1}$)	<LOQ** ($mg\ kg^{-1}$)	LRA*** ($mg\ kg^{-1}$)
Azosxystrobin	1	0.01	0.033	0.01
Chlorpyrifos	1	0.05	0.017	0.01
Epoxiconazol	1	0.01	0.033	0.01

LOD* = Method Detection Limit (Limitofdetection); LOQ** = Method quantification limit (Limitoquantification); LRA*** = Acceptable Reference Limit, from Normative Instruction No. 20, of July 26, 2018.

QuEChERS modified and GC-MS/MS methods detected other pesticides, although values below the detection limit were found. Pesticides, Atrazine, Bentazone, 2,4-D, Bispyribac, Bitertanol, Boscalide, Bromoconazole, Carbaryl, Carbendazim, Carbofuran-3-hydroxy, Carbofuran, Carboxin, Cyanazine, Clomazone, Chloraphenicol, Chlorprofan, Deltamethrin, Diazinone, Difenoconazole, Dimethoate, Fempropathrin, Fempropimorph, Fenarimol, Fenthione, Fipronil, Flutolanil, Imidacloprid, Improvalicarb, Linuron, Malathion, Mecarban, Mepronil, Metalaxyl, Metconazole, Methiocarb-sulfoxide, Metsulfuron-methyl, Mevinphos, Myclobutanil, Monocrotophos, Monolinuron, Oxamyl, Paraoxom-ethyl, Pyraclosthrombin, Pyrazophos, Pyridabem, Pyrimethanil, Pyrimicarb, Pyrimifos-methyl, Prochloraz, Profenophos, Propargito, Propiconazole, Promizamide, Propoxur, Quinoxyphene, Salbutanol, Simazine, Sulfadimethoxine, Sulfamethazine, Sulfathiazole, Tebuconazole, Terbuthylazine, Tetraconazole, Thiacloprid, Thiamethoxam, Tolclophos-methyl, Triadimefon, Triadimentol, Triazophos, Trichlorfon, Trifloxystrobin, Triflumizole and Trimeptopim.

Discussion

Good management practices (GMPs) in aquaculture, prophylaxis and biosecurity is a set of simple and effective measures to optimize the operation of aquaculture establishments and ensure correct and profitable production, respecting GMPs and Stress Mitigating Measures (MMEs) (MAPA, 2019). Besides, for Leung et al. (2020) and Barros, Reis, Campelo, Veras and Brabo (2020) environmental quality is also an essential element in maintaining the health of aquatic organisms, as well as for aquaculture health. At the same time, the different types of rearing systems, extensive, semi-intensive and intensive adopt mitigating measures of GMPs and MMEs to improve fish production and health, which when not well executed predisposes to greater susceptibility to diseases and low productivity. Mainly, when measures such as quarantine, prophylactic baths and inefficient biosecurity measures are not observed (Barros et al., 2020).

Among cultivation systems, the semi-intensive is a low cost and high productivity means of production. However, less than intensive systems due to lower stocking density and production inputs (Barros et al., 2020). Simultaneously, its popularity has increased in the last two decades in several countries around the world, especially among small farmers (Barakat, Mostafa, Wade, Sweet, and El Sayed (2013). It is noteworthy that all properties visited in the Microregion of Zona da Mata practice the semi-intensive system of rearing in excavated tanks with high population densities. Pulkkinen et al. (2010) have showes that high densities can also negatively influence cultivation, promoting reduced weight gain, increased stress factors and reduced growth, immune function and reproduction, causing mortality and susceptibility to disease. In addition, inadequate management with drops in dissolved oxygen levels can lead to reductions in growth rates and/or eventual mortality. In this scenario, the clinical manifestation of diseases and herapeutic treatment and prophylaxis of the culture are expected. Mainly, in establishments that do not adopt measures of good sanitary and biosafety management practices (MAPA, 2019), as observed in 70% of the properties visited.

Therapeutic and/or prophylactic treatment in growing tanks was reported in 55% of the properties, especially in the control of bacterial, mycotic and parasitic etiological agents, with the use of antibiotics being the most mentioned among the fish farmers interviewed. However, the use of these medications, eventually administered in crops, have impacts on environment, as a result of the release of organic and inorganic residues (Ribano et al., 2004). These impacts tend to be more severe if water change is not carried out properly, leading to increased food safety risks and the presence of antibiotic residues in fish that have been treated with them (Okocha, Olatoye and Adedeji, 2018). However, in this study, no antibiotic residues were identified in the musculature of fish farmed in these establishments, by means of chromatography analysis by GC-MS/MS modified method.

However, it should be noted that the indiscriminate use of antibiotics has an impact on environment and can lead to emergence of antimicrobial resistance, both in commensal bacteria from the human and animal intestines, as in bacteria free in the invironment, with the possible dissemination of genes of resistance among diverse bacterial populations (Dunier and Siwicki, 1993; McEwen and David, 2006). As well as the ability to horizontally transfer antimicrobial resistance genes from aquatic environmental bacteria such as *Aeromonas salmonicida* to bacteria such as *Escherichia coli* (Ghenghesh, Ahmed, El-Khalek, Al-Gendy and Klena, 2008). Also, it has been showes that antimicrobial resistance genes can be present in residues products from animal production and can survive for a long period of time in the soil (Aarestrup, 2005; Gasparotto et al., 2020). At the same time, the presence of parasites in tambaqui was reported by the producers, mainly by *Perulernaea gamitanae*, and the use of antiparasitic was mentioned in 17% (5/30) of the properties, mainly in the form of baths based on sodium chloride and the Neguvon® (Trichlorfon). It is known that Trichlorfon was banned in Brazil through Resolution No. 158, of August 18, 2010 by *Agência Nacional de Vigilância Sanitária* [ANVISA].

Lopes, Paraiba, Ceccarelli, and Tornisielo (2006) carried out a study on the toxicological effects of Trichlorfon chemotherapy in *Piaractus mesopotamicus* (pacu) juveniles. The author concluded that Trichlorfon did not cause changes in hematological parameters at sublethal concentrations for a period of 96 hours. However, the chemical caused leukocyte changes in sublethal concentrations, within 96 hours of exposure, with an increase in the amount of lymphocytes and a reduction in the amount of monocytes. Furthermore, the same author found that the administration of Trichlorfon in tambaqui with a high degree of parasite infestation, by ectoparasites of the Argullus and Dolops genus, can favor bacterial infections. Although the use of trichlorfon in parasitic control has been reported, it was not identified in the musculature of fish sent for analysis by chromatography.

However, a sample from a property in the municipality of Alta Floresta D'Oeste, in Rondônia state, was positive for the insecticide Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloropyridin-2-pyridinylphosphorothioate) used in the spraying of animals and cultures, and the following LOD result (0.010 mg kg-1) was obtained. This value was the lowest concentration of the substance under examination that can be detected (Ribani, Bottoli, Collins, and Jardim, 2004). And, <LOQ (0.017 mg kg-1) was the smallest amount of the compound of interest that could be accurately quantified (Ribani et al., 2004). However, according to the PNCRC (MAPA, 2018) the Maximum Residue Limit (MRL) of pesticide Chlorpyrifos in fish musculature is 0.01 mg kg-1. Since the method's Detection Limit (LOD) for this analyte is 0.01 mg kg-1, the concentration of Chlorpyrifos detected in the sample is above acceptable limits, although at a concentration lower than the LOQ (0.033 mg kg-1).

In the survey carried out by the National Plan for the Control of Residues and Contaminants - PNCRC/MAPA (MAPA, 2019), during the period 2013 to 2018, Chlorpyrifos was detected in 1.49% of milk samples and Chlorpyrifos Etil in 0.11% of cattle slaughtered in slaughterhouses with Federal Inspection. The use of these pesticides to protect crops and control ectoparasites in animals can result in the presence of toxic residues in environmental matrices (Mello and Silveira, 2012). In the aquatic environment, pesticides can freely dissolve in water or bind to suspended matter and sediments, and can be transferred to tissues of aquatic organisms, as observed in the tambaqui of this study, as well as to other species, during bioaccumulation processes, resulting in adverse consequences for non-target species.

However, it was not possible to determine the origin of the contamination of Chlorpyrifos residue in the fish musculature, as this fish farming, as well as the other 48 (88.89%) visited, practice polyculture (livestock, aquaculture and agriculture). Furthermore, it is possible that veterinary products containing Chlorpyrifos were administered in the treatment of fish, as the use of antiparasitic drugs was reported in this establishment. It should also be noted that the installation of tanks in lower areas of the property can provide the addition of unintentional dumping, launching, transport, drift or leaching of chemical substances or biological material to the environment, representing a possible source of contamination of residues for fish farming from agriculture (Glibert, 2017).

For Annett, Habibi and Hontela (2014) phytochemicals, when used in agriculture, can reach surface waters, and it is possible to divide them into those that can be transported dissolved in water and those that are transported associated with suspended sediment. Therefore, similar to Chlorpyrifos, we believe that the identification of two fungicides, Azoxystrobin and Epoxiconazol, present in the tambaqui musculature samples from two properties, were transported dissolved in water from the crops to cultivation tanks.

The chemical compound Azoxystrobin (fungicide) (<LOQ) was identified in a fish farming belonging to the municipality of Alto Alegre dos Parecis, RO. Azoxystrobin-based fungicides have been overused due to their broad spectrum, and are potential contaminants of water, soil, food and non-target organisms, affecting animal and human health and generating great concern about their possible effects on organisms (Jiang, Shi, Yu, Chen and Zhao, 2018). In addition, azoxystrobin is the 9th pesticide most found in samples of plant foods, of the 232 pesticides surveyed, according to the PARA report (ANVISA, 2019).

It is worth remembering that Azoxystrobin is a systemic action fungicide of the chemical group of strobilurins that acts through translaminar and lateral mobility with preventive action against biological targets in cotton, rice, banana, potato, coffee, sugarcane, citrus and beans, tomatoes (ANVISA, 2019). Azoxystrobin's mechanism of action consists of inhibition of mitochondrial respiration, resulting in ATP shortage and induction of oxidative stress. In addition, the fungicide acts to control four classes of fungi (Ascomycota, Deuteromycota, Basidiomycota and Oomycota). However, the mode of action of this substance is not specific and can cause adverse effects in other eukaryotic organisms (Rodrigues et al., 2013).

Similarly, Epoxiconazol (<LOQ), a fungicide with systemic action of the chemical groups strobilurin and triazole (Tonin, Reis and Avozani, 2017) was detected in the musculature of tambaqui farmed on a fish farming

in the municipality of Rolim de Moura, RO. The French national agency for food safety, environmental and occupational health (ANSES) has banned the use of this fungicide in the country because of its carcinogenic, mutagenic, reproductive toxic and endocrine function. As in France, England is also banning the use of the product. Concomitantly, Epoxiconazol is a moderate inhibitor of the activity of 17-hydroxylase, responsible for the production of cortisol, as well as a chemical substance that acts by disrupting the endocrine system. Exposure to this substance, considered hormonally active, induces continuous cell proliferation in the ovaries, breast, prostate and uterus and vulnerability to neoplasms (Gowkielewicz et al., 2019).

In both fish farm, both in Rolim de Moura and Alto Alegre dos Parecis, RO, coffee was planted and such fungicides are prescribed for the treatment of Rust and Cercosporiosis in this crop (Silva et al., 2014). In summary, fungicides and agricultural defensives can contaminate aquatic environments by leaching resulting from intense rains. Therefore, farmed fish can be contaminated after short or long term exposure by particles suspended in water or sediment (Mello and Silveira, 2012). Therefore, the contamination of water bodies by this chemical compound represents a risk to both the aquatic ecosystem and public health (Lopes and Albuquerque, 2018). It should also be noted that two fish farm reported the use of fungicides for the treatment of fish, although both properties were not positive for the product in the musculature tambaqui. It was also observed in 54% (29/54) of the fish farm visited use of agricultural defensive directly in cultivation tanks for the control of aquatic plants. Aquatic plants are vegetables that grow in temporary or flooded environments and are characterized by their high growth rate and asexual reproduction capacity. The excessive growth of such plants has harmed the multiple use of water resources, mainly due to anthropogenic action on the environment and the resulting effects, such as excess of nutrients, imbalance in the chain of natural enemies, alteration of the water regime and introduction of exotic species (Glibert, 2017).

Among the main active ingredients used in several countries around the world to control aquatic plants, the following stand out: 2,4-D, diquat, endothal, fluridone, glyphosate and imazapyr (Souza et al., 2017). There is currently no product available with registration for use in Brazil. The fluridone herbicide, which was approved by ANVISA, is currently unregistered (Reid et al., 2019). However, among the producers of this study, the use of Roundup® (Glyphosate) and Flumyzin 500® (Flumioxazine) in the control of aquatic plants were mentioned. Glyphosate is a herbicide used worldwide to control invasive plants and its mechanism of action is through the inhibition of the EPSPS enzyme (5-enolpyruvylshikimate-3-phosphate synthase), which catalyzes aromatic amino acid synthesis reactions (Aarestrup, 2005). However, Glyphosate is rapidly deactivated in natural waters by binding to various cations found in the water and therefore should not be used for submerged weed control.

In Brazil, the chemical control of macrophytes was regulated in July 2015, by Resolution No. 467, which provides for criteria for the authorization of the use of products or agents of physical, chemical or biological processes for the control of organisms or contaminants in surface water bodies (Vivek, Veeraiah, Padmavathi, Rao and Bramhachari, 2016). However, this resolution made it possible to expand the use of chemicals in water bodies, which further motivated studies on effectiveness of herbicides for the control of macrophytes (Reid et al., 2019). Therefore, the use of herbicides to control macrophytes involves several discussions, not only about the physicochemical properties of the product and the efficiency of its use to control macrophytes, although also about the negative impact on ecosystems, the toxicity to aquatic organisms, non-target plants and even humans (Andrada et al., 2020). However, Glyphosate and Flumiaoxazine were not identified in muscle tissue samples from tambaqui, sent for chromatography analysis.

Conclusion

Thus, in this study, eutrophication arising from agriculture was the main means of contamination of fish crops, mainly related to coffee cultivation and the use of systemic fungicides Azoxystrobin and epioxiconazole, although sanitary and biosafety management practices (GMPs) are less than ideal in fish farm visited, such measures did not impact the contamination by drugs residues in the cultive tanks. At the same time, tambaqui (*Colossoma macropomum*) proved to be an important biomarker for environmental contamination. However, more studies must be carried out to investigate the contamination of fish by use of Glyphosate or other pesticides in control of aquatic plants and veterinary products used without dosage for the treatment of illnesses.

In this study were pesticide residues were detected in the muscle tissue tambaqui raised in fish farming in the Microregion of Zona da Mata, Western Amazon. The presence of Azosxystrobin, Epoxiconazol and

Chlorpyrifos in muscle tissue tambaqui samples was detected above the limits acceptable by the National Plan for the Control of Residues and Contaminants in Products from Animal Origin (PNCRC). Consequently, they demonstrate the need for continuous environmental monitoring. In addition to turning on an alert, not only for the health and survival of aquatic species. Although also, for the risk to human populations that consume contaminated fish.

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