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Review

Fatty acids as suitable biomarkers to assess pesticide impacts in freshwater biological scales – A review

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ABSTRACT

The toxic effects associated to pesticides have been for long a concern in terms of environmental and human safety. The recognition of the limitations of numerous, if not all, pesticides in terms of species-specificity, toxicity level, persistency in the environment, influencing its runoff and transport ability, among other characteristics, have been the subject of numerous studies over the years. These have especially focused on the deleterious impacts of such substances in non-target organisms, including potential effects in humans, considering pesticide uptake through the ingestion of contaminated foodstuff. Aquatic ecosystems may be considered the most vulnerable to pesticide contamination, has most toxicants may eventually end up in these systems, if not directly, via indirect inputs. Aquatic communities are, thus, susceptible both to on-point acute contaminations and to exposure to mixtures of different contaminants for longer periods. Impacts of numerous pesticides in non-target aquatic species have been reported and investigated, contributing to the definition of regulations and legislation to prevent harmful effects to the communities. However, in order to assess effectively the effect of different pesticides in organisms, the definition of suitable biomarkers is crucial. As one of the most identifiable effect of pesticides is cell damage induced by oxidative stress, most studies have resorted to the analysis of antioxidant enzymes to assess the impacts of contamination in the organism. Nonetheless, with methodological advances, other molecules have been identified as potentially useful to be used as biomarkers, as fatty acids. These ubiquitous macromolecules in living organisms are major constituents of biological membranes and tissues. Evidence of the impact of xenobiotics on lipid metabolism and composition, as well as the sensitivity of fatty acids profiles to alterations in the homeostasis of organisms, have been arising, supporting the suitability of these macromolecules as biomarkers of toxicants exposure. However, most studies using fatty acids as biomarkers have dwelled mostly on marine ecosystems, disregarding other aquatic systems, as freshwater systems. Given the importance of these systems as providers of numerous services and goods and their role in ecological integrity and support, the present literature review aims to compile and critically review the studies conducted until the present time regarding the use of fatty acids as biomarkers of pesticide exposure assessment in freshwater communities, as well as highlight limitations identified and propose future research.

1. Introduction

The deleterious effects of pesticides to the environment was more deeply investigated since the middle of the 20th century, motivating, as well, the awareness of the general public towards this issue.

However, the recognition of the consequences of the use of pesticides, either herbicides, fungicides, insecticides, has not resulted in an overall decrease of their usage in agriculture and industries worldwide. The leakage, runoff or direct input of pesticides used in these activities contaminates both terrestrial and aquatic ecosystems (including surface and groundwaters) (Sharma et al., 2019). Particular attention has been addressed to the later ecosystems, as transport and spread of the toxicants is easier compared to terrestrial environments (Carvalho, 2017). Dangers of pesticides to aquatic environments have, thus, been identified for most substances, and recommendations and regulations have been reinforced concerning their utilization (Lee, Uyl and Runhaar, 2019; Kristoffersen et al., 2008). Nonetheless, best practices are not always applied, and the effect of the toxicants in non-target organisms, although often identified, lacks research and more thorough measures, legislation and regulation to prevent unreversible alterations in

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communities and ecosystems in general, provoked by pesticides' toxic effects, potentially disrupting the ecological balance of the contaminated environments (Moraes et al., 2007). Pathological and biochemical processes may be disturbed, including changes in energy metabolism (Villarroel et al., 2009), neurotransmission impairment (Chebbi and David 2009) and oxidative stress. The latter two are the most well-studied toxicological mechanisms induced by exposure of organisms to pesticides. Such parameters are of biological and ecological relevance, particularly in aquatic ecosystems, given that these environments may accumulate various contaminants that potentially induce oxidative stress in organisms (Kelly et al., 1998).

Exposure to stressors generally induces changes in the normal repartition of an organisms' energy. Most organisms in non-stress conditions use their energy storages and sources for basal metabolism, growth and reproduction. In stress conditions, caused, for example, by exposure to a toxicant, organisms' energy allocation may be altered in order to cope with the induced stress (Jeon et al., 2013). A decrease in energy reserves will take place and organisms may have to converge their energy outflows to a single mechanism among the above-mentioned (Sancho et al., 2009), in an effort to guarantee individual or species survival.

Oxidative stress induced by pesticides, through an increase in the formation of reactive oxygen species (ROS), may lead to biochemical, cellular and physiological changes in the exposed organisms. Free radicals may lead to lipid peroxidation, changing the constitution of the biological membranes, as well as oxidative injury to DNA and proteins (Kelly et al., 1998). The cells of every living organism have protective mechanisms against oxidative stress, balancing the redox status and trying to maintain cell homeostasis.

Fatty acids (FA) are in the basis of lipid molecules and have a very important role in all living organisms, being one of the main constituents of cellular membranes. FA are accumulated as energy reserves and transported and metabolized for different final purposes. FA are a large family of components, varying in length, unsaturation degree and substitute groups. Polyunsaturated fatty acids (PUFA), for example, are an essential class of FA with vital functions such as maintaining cellular membrane characteristics, being hormone precursors and prevent or delay several diseases (e.g. cancer, cardiovascular diseases, atherosclerosis, autoimmune diseases), "dysfunctional" behaviours (e.g. episodes of extreme violence), neurological diseases (e.g. schizophrenia, Alzheimer's disease) and pain resulting from rheumatoid arthritis, symptoms of eczema and psoriasis (Connor, 2000; Simopoulos, 2002, 2009). Although PUFA are almost exclusively synthesized by plants, they are essential to animals. Thus, animal uptake PUFA mainly through diet, and then metabolize and alter the molecules into others by elongation and desaturation enzymatic-mediated mechanisms (Brett and Muller-Navarra, 1997).

The exposure to pesticides may affect fatty acid composition of organisms by potentiating lipid peroxidation, as previously mentioned, or interfering with lipid metabolism, which can be evaluated through the assessment of fatty acid profiles. In the last few years, fatty acid content of organisms has grown as a research interest area, and significant changes in fatty acid composition resulting from pesticide exposure have already been documented in literature (Galhano et al., 2011; Lal and Singh, 1987; Rosas et al., 1980)). Variations in total fatty acid content or in the percentage of a given FA class may be indicative that a pesticide is affecting the organisms' normal metabolism, either due to a change in the production mechanisms of FA in the organisms that produce them (mainly autotrophic organisms), diets or to an abnormal processing of the ingested FA (in the case of consumer trophic levels).

There is a vast number of pesticides developed and applied by humans in bare environments (Aktar et al., 2009; Lazartigues et al., 2013; Margni et al., 2002; Maugh, 1978). Most pesticides are a mixture of active substances – chemical compounds that are biologically active and produce the wanted effect – and other compounds that can have indirect functions, as facilitators of spreading or bioaccumulation of the active substance. Therefore, pesticides have numerous structures and properties, including different targets and action mechanisms. Pesticides may be classified through different parameters, for example considering the substance target (herbicides, insecticides, fungicides, bactericides, etc.) or chemical class. Regarding the latter form of classification, organophosphorous pesticides (OPPs) and carbamates (CMs) are among the most common and widely used pesticides. Despite their overall rapid biodegradation, if applied correctly, the large spectrum of action, runoff and high toxicity of some of these products to unintended species, pose a serious concern and rise the necessity to take into consideration the potential ecological impacts of such products (Aktar et al., 2009; Lazartigues et al., 2013). Parathion, malathion, methyl parathion, chlorpyrifos (CP), diazinon, dichlorvos, phosmet, tetrachlorvinphos, triazophos, oxydemeton and azinphos methyl (AZM) are examples of OPPs. Organochlorine pesticides (OCPs) are the most toxic and persistent pesticides on the environment. This class of pesticides includes aldrin, chlordane, chlordecone, dieldrin, endrin, heptachlor, hexachlorobenzene, alpha and beta-hexachlorocyclohexane (HCH), lindane, mirex, pentachlorobenzene, toxaphene, dichlorodiphenyltrichloroethane (DDT) (Sankararamakrishnan et al., 2005), all of which have a great capacity of bioaccumulation in biological membranes, magnifying along food chains (Van Dyk and Pletschke 2011). Triazine pesticides are a third class of pesticides to be taken into consideration, constituted predominantly of herbicides able to selectively inhibit the electron transport in photosynthesis, thus representing an environmental concern regarding non-target photosynthetic organisms. Although the three classes comprise the majority of the most commonly used pesticides, numerous other compounds, of different classes are equally common, and will be mentioned and described along the document.

Over the years, especially in more developed countries, highly toxic and persistent pesticides have been legally banned and replaced with others that present faster degradation and more species-specific. However, and namely in developing countries, the low cost/efficacy ratios of some more toxic pesticides rule over the potential ecological impacts they pose, sometimes leading to the use of already banned substances. Although more recent pesticides present improvements in terms of ecological damage, they still carry serious ecotoxicological issues – even the less toxic pesticides may derive negative impacts on ecosystems in many ways, that will be later discussed. Therefore, there is an increasing need to understand the processes that generate specific responses by non-target organisms.

2. Current trends in the use of fatty acids to assess the response of non-target organisms to pesticides

This literature review aims to provide a critical perspective and evaluation of the use of fatty acid profiles in freshwater organisms to determine and assess the response of aquatic communities to pesticides present in the environment. The research publications selected to produce this critical review assessed potential responses to the presence of pesticides at different classes in the fatty acid profile of non-target freshwater organisms exposed to the contaminants.

The review highlights the lack of information regarding the use of fatty acid profiles as tools to identify and assess the response to pesticides by freshwater organisms, especially compared to marine counterparts or other biomarkers (e.g. enzymes). Nonetheless, most studies were able to identify a correlation between changes in the fatty acid profile and pesticide exposure, and if the impact in the fatty acid profiles was direct (the pesticide provoked some change in the organism that led to a change in the FA profile) or indirect (the food source of the organism under study was affected, therefore influencing the FA profile of its consumer). Overall, the use of fatty acids as biomarkers to study the toxic response of non-target species to pesticides in freshwater ecosystems appears to be a promising field of research with potential to become a widely used tool with wide application and reproducibility.

3. Fatty acids as suitable biomarkers of pesticide contamination

Biomarkers are biological parameters that are measurable and indicate a change or presence in a considered biological system. They have been divided into three classes: (a) biomarkers of exposure, that allow the recognition and measurement of xenobiotics inside an organism, their metabolites or interaction products, (b) biomarkers of effect, which allow the quantification of an altered state of the biological system in observation, by the measurement of certain endogenous constituents, such as biochemical or physiological changes, (c) biomarkers of susceptibility, that indicate a response of the organism to a given xenobiotic, like changes in the genetic expression of proteins or receptors that affect the susceptibility to such compounds, also allowing a better understanding of the response variations of different organisms (NRC, 1987; van der Oost et al., 2003). However, this division is not clearly defined, especially between biomarkers of effect and exposure, since biomarkers' levels change in response to the exposure to a contaminant. This means that the same biomarker can be classified as a biomarker of effect or exposure, depending on its usage. If used to assess the relation between the exposure to a contaminant and an internal uptake of such contaminant, it should be classified as a biomarker of exposure. If the goal is to observe and measure biochemical alterations, that affect the organisms' wellbeing, due to the exposure and accumulation of that toxicant, the used biomarkers should be classified as biomarkers of effect (van der Oost et al., 2003).

The ecotoxicology of pesticides in non-target species is many times unknown and hard to predict, once these compounds were not designed to cause a response by such organisms but are highly likely to do so. Thus, the use of biomarkers to assess biochemical and physiological responses to pesticides by such organisms is of extreme importance, but the choice of the right biomarker is not always simple, given that the mechanisms of ecotoxicity are rarely fully understood. So, there is a need to choose biomarkers that evidence the final potentially harmful effects in the organisms, and thus a specific response by the individuals, and that are reproducible and accurate.

The generation of reactive oxygen species (ROS) and consequent oxidative stress is another almost ubiquitous response of non-target species to pesticide exposure (Sayeed et al., 2003; Kavitha and Rao, 2008). ROS exists in cells of all organisms in normal conditions, produced mainly in mitochondria, chloroplasts and peroxisomes, and are needed for certain cell functions, as long as the production and elimination rates are balanced to guarantee cell redox homeostasis. If the oxidative stress induced by a contaminant, by the disruption of ROS balance in cells, is too high or too persistent, the antioxidant response will not be able to fully compensate ROS over-production (Dröge, 2002), affecting many compounds of the cell, causing lipid peroxidation, particularly dangerous for the integrity of biomembranes, as structural lipids may be affected.

Lipids such as fatty acids, triglycerides, phospholipids, to name a few, are major constituents of biological membranes and tissues of living organisms. Freshwater organisms' fatty acid and phospholipid profiles are dependent not only on the organisms' lipid metabolism – biosynthesis and oxidation -, but also on the lipids ingested through nutrition. Therefore, the mentioned lipid profiles are subjected to dietary and seasonal changes (Gonçalves et al., 2012; Taipale et al., 2009).

Moreover, evidences of the response to the presence of xenobiotics in lipid profiles of freshwater organisms have been reported, namely affecting lipid metabolism (Lal and Singh, 1987) and fatty acid and phospholipid composition (Galhano et al., 2011; Singh and Singh, 2007; Zhong et al., 2012).

Studies supporting the relevance of the use of fatty acids as biomarkers of pesticide exposure have gained importance in recent years. Here is compiled the studies addressing the response to the pesticide exposure on the fatty acid profile of freshwater species and potential effects on food webs.

3.1. Photosynthetic organisms

As photosynthetic organisms are at the base of most trophic chains, impacts on their integrity may compromise whole food webs with repercussions hard to fully predict. Numerous studies have reported the response of photosynthetic communities to pesticides exposure, but few have used fatty acids as biomarkers of induced stress. Photosynthetic organisms present high content of PUFA and highly unsaturated fatty acids (HUFA), in particular the omega-3 fatty acid eicosapentaenoic acid (EPA, 20:5*n*-3) (Ackman et al., 1964; Hamilton et al., 2014). Changes in the fatty acid classes' ratios may mean a response of the organisms to some alteration, namely pesticide exposure. As the input of the FA produced by primary producers is truly important for the fitness of primary consumers, such changes may mean a disequilibrium in a food chain's integrity.

The response of phytoplanktonic species to OPPs was assessed by Galhano et al. (2011) and Kumar et al. (2014) in *Nostoc muscorum* and *Chroococcus. turgidus*, respectively. The authors reported similar results concerning saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) of both species exposed to different pesticides of the same class: *N. muscorum* exposed to Ordam® (the commercial formulation of molinate) and *C. turgidus* exposed to chlorpyrifos showed a significant decrease in SFA content after exposure to both pesticides; *C. turgidus*, however, presented SFA species that were not present in control organisms (such as tridecanoic, C13:0, myristic C14:0, heneicosanoic, C21:0 and behenic C22:0 acids). An overall increase in MUFA content was observed in both species, especially concerning palmitic acid in *N. muscorum* and oleic acid (C18:1) in *C. turgidus.* The response to pesticide exposure concerning poly and highly unsaturated fatty acids, however, differed between the two species, with overall PUFA and HUFA increasing in *N. muscorum* (in particular EPA and alpha-linolenic acid, C18:3*n*-3) with increasing pesticide concentrations but decreasing in *C. turgidus* (especially EPA and linoleic acid, C18:2). The justification for both results comes from the same premiss: pesticide exposure induced oxidative stress in the organisms, caused by ROS generation induced by pesticide exposure. In order to cope with stress, both cyanobacteria reacted in different ways. Oxidative stress decreases membrane fluidity, compromising cell function. The desaturation of fatty acids triggered in *N. muscorum* was, then, a means to increase membrane fluidity to compensate the effect of pesticide exposure. However, ROS also damages polyunsaturated fatty acids, which was reported in *C. turgidus*. In this case, the cyanobacteria produced SFA that were not present in control organisms as to decrease the degree of saturation, to prevent damage from oxidative stress. Yet, it is worth noticing that the concentrations of the pesticides used in the referred cyanobacteria were quite different (as shown in Table 1), which prevents a better correlation between the results and determination of the most toxic. Nonetheless, it is possible to clearly observe an impact of pesticide exposure in both species, that produced different copping responses.

Other pesticides, of different classes, were used by several authors to assess the response of phytoplanktonic species to the exposure to these contaminants, as described below.

Bessa da Silva et al. (2016) studied the response of the phytoplankton species *Raphidocelis subcapitata* to the pesticide Prowl®, a commercial formulation of pendimethalin, a dinitroaniline herbicide used for annual grasses and weeds control. The total content of SFA, PUFA and HUFA, although higher in control organisms, showed no significant differences between control and exposed organisms, and only MUFA content suffered a significant decrease in organisms exposed to the contaminant. Nonetheless, a significant decrease in the content of certain fatty acids of organisms exposed to the pesticide was reported, namely of undecylic (C11:0), pentadecanoic (C15:1), palmitic (C16:0) and oleic (C18:1n9) acids. Despite the differences observed, the authors concluded that the fatty acid composition of the studied green algae was quite similar between control and exposed groups, with the essential linoleic and alpha-linoleic acids present in both cultures.

Table 1
Summary of alterations in FA profiles of freshwater species after exposure to pesticides.

Type of Organism	Species name	Analysed part of the organism	Pesticides	Pesticide Mode of Action	Conditions of exposure (concentration range/ time)	SFA	MUFA	PUFA	HUFA	Ref.
Cyanobacteria	*Chroococcus turgidus*	Whole cell	Chlorpyrifos	Induction of oxidative stress and subsequent cellular components damage	6 mg.l^{-1} 7 days	↘ C8:0; C10:0; C11:0; C18:0; C22:0; C24:0 ↗C13:0; C14:0; C21:0; C22:0	↘C20:1 ↗C18:1	↘C18:2 ↘Total PUFA	↘C20:5	Kumar et al., (2014)
	Nostoc muscorum	Whole cell	Molinate	Induction of oxidative damage	[0.75 – 2] mM 72 h	↘C14:0; C16:0; C20:0; C22:0; C24:0	↗C16:1	↗C18:3	↗C20:5	Galhano et al., (2011)
Microalgae	*Raphidocelis subcapitata*	Whole cell	Pendimethalin	Induction of oxidative damage	[0.294 – 10.000] μg.l^{-1} 96 h	↘C11:0; C16:0	↘C15:1; C18:1	N/1	N/1	Bessa da Silva et al., (2016)
Diatoms	*Gomphonema gracile*	Whole cell	Diuron	Inhibition of photosystem II	10 μg.l^{-1} 7 days	↘C14:0; C16:0	↗Total MUFA ↗C18:1	↘Total PUFA ↗C16:2; C16:3 ↘C18:2	↘Total HUFA↘C18:4; EPA	Demailly et al., (2019)
			S-metolachlor	Inhibits synthesis of chlorophyll, proteins, fatty acids, lipids and organism growth	10 μg.l^{-1} 7 days	N/1	N/1	N/1	N/1	
Cladocerans	*Daphnia longispina*	Wholecommunity	S-metolachlor	Disrupts protein synthesis, and FA metabolism	[3.33–8.24] mg.l^{-1} 21 days	N/1*F0N/1*F1	↗F0(-CTL) F1:(-CTL) O↘ M↗	N/1*F0N/1*F1	↘in F0(-CTL) ↘in F1(-CTL)	Neves et al., (2015)
	Daphnia magna	Whole community	Pendimethalin	Induction of oxidative damage	Cladocerans were fed with algae exposed to pesticide at [0.294 – 10.000] μg.l^{-1} 96 h	↘C11:0; C16:0	↘C15:1; C18:1	N/1	N/1	Bessa da Silva et al., (2016)
Macro-invertebrate	*Biomphalaria alexandrina*	Soft tissues	Diazinon	Inhibition of AChE activity	[0.5 – 4] ppm 24 h	↗C12:0;C14:0; C17:0; C18:0 ↘ C8:0; C9:0; C15:0; C16:0	↘C18:1; C14:1; C16:1	↘C18:2; C18:3	N/A	Bakry et al., (2013)
		Soft tissue	Profenfos	Inhibition of AChE activity	[0.5 – 4] ppm 24 h	↗C12:0; C14:0; C17:0; C18:0	↘C18:1; C14:1; C16:1	↘C18:2; C18:3	N/A	
	Asellus aquaticus	Whole body	Epoxiconazole	Inhibits cell biosynthesis	15 μg.l^{-1}	↘ Total SFA (~-60%)	↘ Total MUFA	↘ Total PUFA	N/A	Feckler et al., (2016)
Fish	*Anguilla japonica*	Dorsal muscles; Abdominal muscle; Tail muscle; Liver; Abdominal Fat	DDT (found) HCH (found) PCB (found)	Deregulates neurons ion channels	N/A	N/A	N/A	N/A	↗(DDT, PCB): DHA, EPA	Geng et al. (2015)
	Oreochromis mossambicus					N/A	N/A	N/A	↗(DDT, PCB): DHA, EPA	
	Ctenopharyngodon idellus					N/A	N/A	N/A	↗(DDT, PCB): DHA, EPA	
	Ctenopharyngodon idellus	Liver; Muscle; Gills; Skin; Intestine; Bladder; Eye; Fat; Kidney	DDT (found) HCH (found)	Deregulates neurons ion channels	N/A	↗SFA	↗MUFA	↗PUFA↗C18:2; C20:3	N/A	Zhang et al., (2019)
	Hypophthalmichthys molitrix					↗SFA	↗MUFA	↗PUFA↗C18:2; C20:3	N/A	
	Cyprinus carpio					↗SFA	↗MUFA	↗PUFA↗C18:2; C20:3	N/A	
	Aristichthys nobilis					↗SFA	↗MUFA	↗PUFA↗C18:2; C20:3	N/A	
	Elopichthys bambusa					↗SFA	↗MUFA	↗PUFA↗C18:2; C20:3	N/A	
	Silurus asotus					↗SFA	↗MUFA		N/A	

(*continued on next page*)

Table 1 (*continued*)

Type of Organism	Species name	Analysed part of the organism	Pesticides	Pesticide Mode of Action	Conditions of exposure (concentration range/ time)	SFA	MUFA	PUFA	HUFA	Ref.
								↗PUFA↗C18:2; C20:3		
	Channa punctatus	Liver; Muscle	Endosulfan	Ion channel inhibitor and ATPase deregulator	8.1 µg.l^{-1} 96 h	N/A	N/A	↘ General PUFA and HUFA		Sarma et al. (2015)
	Barbus conchonius	Liver	Endosulfan	Ion channel inhibitor and ATPase deregulator	6.62 ppb 4 weeks	↗ General FFA				Gill et al., (1991)
	Clarias batrachus	Liver; Plasma; Gonads; Muscle	Lindane	Neurotoxicity	[2–8]ppm 4 weeks	↗General FFA in female and male liver ↗General FFA in female plasma; ↘General FFA in male plasma ↘General FFA in ovaries; N/1 in testes ↘General FFA in female muscle; N/1 in male muscle				Lal and Singh (1987)
			Malathion	Inhibition of AChE activity	[1–4]ppm 4 weeks	↗General FFA in female and male liver ↗General FFA in female plasma; ↘General FFA in male plasma ↘General FFA in ovaries; ↗General FFA in testes ↘General FFA in both sexes muscle				

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FFA, free fatty acids. N/A, not available;
"↗" = increase of FA relatively to increase of pesticide concentration; "↘" = decrease of FA relatively to increase of pesticide concentration; "N/1" = no significant changes;
"-CTL" = except control; "F0" = phase 1: offspring/neonates 1 + mothers 0; "F1" = phase 2: offspring/neonates 2 + mothers 1; "O" = offspring;
"M" = mothers; "N/1*" = irregular changes.

The response of the freshwater diatom *Gomphonema gracile* to diuron, a phenylurea pesticide, and S-metolachlor, of the anilide class, were assessed by Demailly et al. (2019). As expected, the FA profile of *G. gracile* is mostly composed of PUFA and HUFA, with special highlight to the essential fatty acids EPA and arachidonic acid (ARA, C20:4*n*-6). At the exposure to S-metolachlor was not observed alteration at the content or profile in FA of the diatom. On the other hand, at diuron exposure, a decrease in HUFA content was observed, while MUFA content increased. This observation may be explained by an alteration in lipid metabolism, that shifts from the production of membrane lipids to lipid storage. The decrease in PUFA and HUFA content, similarly to the observed in Kumar et al. (2014), previously mentioned, may be derived by an inhibition of desaturase enzymes involved in the synthesis of PUFA and MUFA (Filimonova et al., 2016, 2018).

The results mentioned in this section is provided in further detail in Table 1.

3.2. Zooplankton

Studies using fatty acids as biomarkers to assess the response of zooplankton species to pesticide's exposure are scarce. Zooplankton species, as most animal species, reflect in their fatty acid profile that of their food sources according to the slogan "we are what we eat" (Chen et al., 1995). Therefore, the use of fatty acids as biomarkers in zooplankton species is useful not only to identify changes in the species' fitness, response to environmental conditions and so on, but also to make inferences about the food source.

The experiment conducted by Bessa da Silva et al. (2016) regarding the response of phytoplankton species to Prowl®, already mentioned above, allows to assess the food quality of zooplankton species, in this case the cladoceran species *Daphnia magna*, that feeds on the phytoplankton species *Raphidocelis subcapitata*. The changes in the FA profile of *D. magna* were consistent with the changes observed in the algae, further supporting the fact that the animal species reflect on their fatty acid profile that of the food source.

Cladocerans are known to obtain almost the total amount of their constituent fatty acids through diet, being the *de novo* production of FA almost inexistant (Goulden and Place, 1993). This fact was further supported by Neves et al. (2015), in a study on the effect of S-metolachlor, which has been above mentioned as impacting phytoplankton species, in the fatty acid profile of *Daphnia longispina*, performing laboratory toxicological experiments. Although a slight increase in all FA classes was observed in organisms exposed to the toxicant, the authors reported that this was not a significant change compared to control organisms (not exposed to S-metolachlor). These results are consistent with literature regarding the action mode of the pesticide, as it targets metabolic pathways of FA synthesis.

The use of fatty acids as biomarkers in freshwater zooplankton species is potentially very interesting to identify indirect toxic effects of pesticides in freshwater systems, a matter often difficult to predict and assess in environmental relevant conditions.

The experimental results above mentioned are expressed in detail in Table 1.

3.3. Macroinvertebrates

Macroinvertebrates are also known to reflect the fatty acid profile of their food sources. However, and interestingly, studies have reported a more evident impact of pesticides in macroinvertebrates compared to other animal groups, as zooplankton, for example (Macchi et al., 2018; Muirhead-Thomson, 1987).

Studies regarding the response of macroinvertebrates to pesticides' exposure are considerably more abundant regarding marine organisms compared to freshwater organisms. Considering the ecologically vital role of macroinvertebrates in freshwater trophic webs and the vulnerability of freshwater systems to contamination by pesticides, studies regarding the response to such toxicants on these organisms are of extreme importance (Macchi et al., 2018).

Bakry et al. (2013) assessed the response of the freshwater snail *Biomphalaria alexandrina* to OPPs diazinon and profenfos. Both pesticides altered significantly the FA profile of the exposed specimens compared to control groups, showing a significant decrease in SFA (particularly C8:0, C9:0, C15:0 and palmitic acid C16:0), MUFA (in particular C14:1, palmitoleic acid, C16:1 and oleic acid, C18:1) and the PUFA linoleic acid, C18:2. It is interesting to highlight that palmitoleic and oleic acids, two most common FA in animals, were completely absent in exposed animals. On the other hand, exposure to the toxicants increased the saturated fatty acids C12:0, C14:0, C17:0 and C18:0 content, compared to the results obtained in control snails. The reduction in long-chain fatty acids, both saturated and unsaturated, may be due to the allocation of energy from these compounds to compensate a decrease in glucose metabolism, triggering other lipids hydrolysis and FA oxidation (De Leo et al., 2010). Similarly to the reported in other species, PUFA decrease may have been due to alterations in the species' lipid metabolism, as the pesticides may have impaired the activity of elongation and desaturase enzymes, reducing the production of PUFA. Moreover, lipid peroxidation caused by the action of ROS species, generated by the oxidative stress induced by pesticide presence, that snails' antioxidant mechanisms were unable to effectively eliminate, may also have contributed to the decrease in unsaturated FA.

Feckler et al. (2016) assessed the response of the shredder *Asellus aquaticus* to the fungicide epoxiconazole either through a direct or an indirect pathways, the latter considering the shifts in the microbial communities affected by the fungicide in which the invertebrate feeds. Epoxiconazole is widely used for the protection of crops, but frequently found in surface waters and other fields through water run-off. The FA composition of *A. aquaticus* presented significant differences between the experimental groups compared to the control, with respect both to direct and indirect pathways of the pesticide's impact. Saturated fatty acids content of *A. aquaticus* presented a reduction of approximately 60% after exposure to epoxiconazole; MUFA and PUFA also showed a reduction, possibly due to the oxidative stress and consequent lipid peroxidation caused by pesticide presence, as previously argued. The downward trend of unsaturated fatty acids may pose a serious issue concerning the decline of essential PUFA, especially ARA (C20:4) and EPA (C20:5), and their precursors, which play vital roles in the animal's development.

The response of non-target macroinvertebrates to pesticides provide evidence, once more, of the impacts of pesticides on non-target organisms through direct ways. In this groups' case, the use of fatty acids as biomarkers of pesticide-induced stress and impacts proves to be useful not only to infer the quality of the organisms' food sources, but also the response of the macroinvertebrates to chemical stressors. As these organisms are relatively easy to sample and ecologically relevant to freshwater ecosystems integrity and function, the use of FA as biomarkers comes as a useful tool to assess the response of freshwater macroinvertebrates communities and allows inferences and prediction regarding the rest of the trophic web.

More detailed information about the experimental results obtained in the studies mentioned in this section is provided in Table 1.

3.4. Fish

Research on the response of fish species to pesticides is of particular importance for numerous reasons. On one hand, it may consider the group's relevance in terms of human nutrition and economic activities. On the other hand, the fact that most pesticides are known to bioaccumulate and even biomagnify along food chains, and as fish occupy higher trophic levels, may reflect not only the harmful impacts of pesticides and the response of non-target species such as with economic importance, but also allow inferences regarding the state of whole trophic webs.

Organochlorine pesticides are known to bioaccumulate along food chains and are of particular concern, due to the aggressive impacts of some formulations. This group of pesticides includes the banned substance DDT (Dichlorodiphenyltrichloroethane), HCH (*beta*-Hexachlorocyclohexane) and numerous PCBs (Polychlorinated biphenyls). The accumulation of the referred toxicants and potential correlations with FA content were investigated in the freshwater fish species river eel, *Anguilla japonica*, tilapia, *Oreochromis mossambicus*, grass carp, C*tenopharyngodon idellus,* silver carp, *Hypophthalmichthys molitrix*, common carp, *Cyprinus carpio*, bighead carp, *Aristichthys nobilis*, yellowcheek carp, *Elopichthys bambusa* and catfish, *Silurus asotus* (Geng et al., 2015; Zhang et al., 2019), relevant species for consumption and environmentally relevant due to their different food habits.

Relevant concentrations of DDT and HCH were found in all fish species, while PCBs content was only assessed for river eel, tilapia and grass carp (Geng et al., 2015). The authors took particular interest on correlations between pesticide concentrations and the essential fatty acids EPA and docosahexaenoic acid (DHA, C22:6), as the two fatty acids are among the main aspects to assess the quality of fish as a human food source. In fact, a significant and positive correlation between PCB and DDT concentration and the essential fatty acids content was found by Geng et al. (2015) in river eel, tilapia and grass carp – the increase of contaminant concentrations was accompanied by an increase of EPA + DHA content – but no correlation was found regarding HCH. Zhang et al. (2019) found a significant positive correlation of SFA, MUFA and PUFA (in particular C18:2*n*-6 and C20:3*n*-3) with DDT and HCH in the five carp species (grass carp, silver carp, common carp, bighead carp, yellowcheek carp) and catfish. DHA and EPA correlation with the tested pesticides was not particularized by Zhang et al. (2019).

Higher contaminant pollutant concentrations, together with higher fatty acids contents, were found in the carnivorous fish species in both studies, due to their higher trophic level, susceptible to greater bioaccumulation of the contaminants compared to organisms of a lower trophic level, reflecting the dangers of bioaccumulation effects of pesticides along food chains. Both studies highlighted and discussed the paradox involving fish consumption by humans: if, on one hand, fish are one of the main sources of fatty acids to humans – PUFA and HUFA, in particular the omega-3 essential fatty acids EPA and DHA – on the other hand, being in general animals of high trophic levels, the concentration of accumulated pesticides may pose serious health risks considering the intake of such pollutants by humans.

Another relevant OCP pesticide of common use is endosulfan, a fairly water-soluble substance used in agriculture to combat a wide variety of insects. Endosulfan comprises two isomers – α and β – the first being significantly more toxic but the second persisting longer in the environment (β -endosulfan may last for 2 years in the environment) (Devi et al., 1981; McEwen and Stephenson, 1979). Endosulfan used is currently banned from most European countries, being highly toxic to fish communities (EFSA, 2011). However, the substance was for long considered safer than other OCPs, regarded to degrade quickly and not bioaccumulate in organisms (McEwen and Stephenson, 1979). Endosulfan acts at the nervous system level, interfering with the dopaminergic system (Anand et al., 1985), potentially affecting all animals, and not only its target species.

The response of freshwater fish species to endosulfan exposure regarding their fatty acid profiles were assessed in the rosy barb (*Barbus conchonius*) (Gill et al., 1991) and in the spotted snakehead fish (*Channa punctata*) (Sarma et al., 2015). The two studies showed somewhat different results: considering liver FA content, rosy barb specimens showed an increase in FA content compared to control organisms, and increasing FA content with increasing exposure time; spotted snakehead fish, on the other hand, presented a significant decrease in liver FA content after 96 h of exposure. The FA content of muscle tissue in the rosy barb was not assessed, however, total lipid content results were consistent with the ones found in the spotted snakehead fish, showing a significant decrease with exposure. At the muscle tissue FA content of the latter species was also significantly decreased with pesticide exposure and it would have been interesting if the same had been assessed for the rosy barb. While (Sarma et al., 2015) conducted a 96 h experiment, Gill et al. (1991) subjected fish to endosulfan-contaminated medium for up to 4 weeks. It is interesting to note that the FA content of *B. conchonius* significantly increased in the liver of the fish after 2-weeks exposure to the toxicant, and total lipid content also increased in the muscle tissues after that period (although still significantly lower compared to control organisms). The complete FA profile of *C. punctata* could not be performed, nonetheless, results showed a reduction in unsaturated fatty acids, which could be explained by the use of fatty acids to match energy demands required by the stress induced by the pesticide (Bantu et al., 2013).

Further studies to test the influence of endosulfan exposure for longer periods – lacking in the study conducted by (Sarma et al., 2015) –, concerning the FA content of muscle tissue – lacking in the study by Gill et al. (1991) – , would be interesting to better support the advantageous of using fatty acids as biomarkers to assess responses to acute toxicity of endosulfan exposure and long-term exposure.

Lal and Singh (1987) studied the response of both sexes of freshwater catfish *Clarias batrachus* to two concentrations described as safe and sublethal of the OCP Lindane (γ-BHC, gamma isomer of hexachlorocyclohexane) and the OPP malathion. Overall, lindane exposure resulted in an increase of liver FA in both sexes. Contrary responses between sexes were reported for plasma FA, with a significant increase concentration in female fish and decrease in male fish, and for gonads, where only the sublethal concentration of lindane decreased significantly the ovarian fatty acid content, not affecting testes FA content. Muscular fatty acid content was unchanged in males exposed to both concentrations and, once more, the sublethal concentration of the toxicant decreased the FA content of females' muscle. Exposure to malathion produced similar results in both sexes regarding the liver, where FA concentration significantly increased with exposure to the pesticide, and significantly decrease in muscle. Differences among sexes were found regarding plasma FA, whose concentration significantly increased in females, but decreased in males, and in gonads, with reduced FA concentrations in ovaries, while FA concentration in testes increased.

These results may be compared with the ones reported, and previously discussed, regarding endosulfan exposure, considering the increase of FA content in some tissues after long-term exposure to the contaminant as reported by Gill et al. (1991), and the decrease in others, similar to the observed by the same authors and (Sarma et al., 2015). In lindane and malathion studies, the authors attributed the decrease of FA content in some tissues to a higher demand of energy, similarly to the other authors, or to a potentially impaired transportation of the macromolecules from fish liver – where FA concentration increased with exposure to both pesticides – to other tissues. Regarding the results observed in male *C. batrachus* the increase of FA in testes was, however, hypothesized to be due to a reduction in oxidation or utilization of FA due to the inhibition of the gonads' activity by the pesticide.

More detailed information about the experimental results obtained in the studies concerning the fatty acid profile of different fish species subjected to pesticide exposure is provided in Table 1.

4. Future perspectives

The present review highlights that the number of studies regarding the use of fatty acid profiles as biomarkers to assess pesticide exposure of freshwater species is truly scarce. Nonetheless, the studies conducted on the topic concluded that fatty acids may be a resourceful indicator of oxidative stress in organisms exposed to pesticides, and give an insight on how the substances affect different tissues, mainly through lipid peroxidation or tampering with lipid metabolism, potentially harming biomembranes and metabolic processes. Thus, the continuity of the study of FA as biomarkers of pesticide-induced stress may provide valuable and accurate information on the different mechanisms that organisms activate to control the harmful action of pesticides, other than the well-known set of other biomarkers, as antioxidant enzymes. It would also be expected, and desirable, that the numerous studies assessing the response of species from different trophic levels to the presence of various pesticides encourage the production of pest-control substances more species-specific, less toxic, less persistent substances, lowering their ecological impact and strengthening the regulation for pesticide use, in order to preserve ecosystems and prevent unknown effects on non-target organisms, with consequences potentially beyond our current understanding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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