ENVIRONMENTAL POLLUTION AND TOXIC SUBSTANCES: CELLULAR APOPTOSIS AS A KEY PARAMETER IN A SENSIBLE MODEL LIKE FISH

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Abstract

The industrial wastes, sewage effluents, agricultural run-off and decomposition of biological waste may cause high environmental concentration of chemicals that can interfere with the cell cycle activating the programmed process of cells death (apoptosis). In order to provide a detailed understanding of environmental pollutants-induced apoptosis, here we reviewed the current knowledge on the interactions of environmental chemicals and programmed cell death. Metals (aluminum, arsenic, cadmium, chromium, cobalt, zinc, copper, mercury and silver)as well as other chemicals including bleached kraft pulp mill effluent (BKME), persistent organic pollutants (POPs), and pesticides (organo-phosphated, organo-chlorinated, carbamates, phyretroids and biopesticides) ~~and cyanobacterial toxins (microcystine, anatoxins-a and saxitoxins, nodularin and cylindrospermopsin, lipopolysaccarides)~~ were evaluated in relation to apoptotic pathways, heat shock proteins and metallothioneins. Although research performed over the past decades has improved our understanding of processes involved in apoptosis in fish, yet there is lack of knowledge on associations between environmental pollutants and apoptosis. Thus, this review could be useful tool to study the cytotoxic/apoptotic effects of different pollutants in fish species.

Key words: Apoptosis, Environmental Water Pollutants, Chemicals, Water Toxicity, Fish.

Introduction

Controlled cell death is an evolutionary conserved process that plays a key role in the development and homeostasis of all metazoan animals (Janz et al., 2001). Tissue homeostasis requires a sensitive balance that is maintained between cell renewal and cell death, and homeostatic cell deletion is a well-controlled mechanism regulated by apoptosis (Beere et al., 2000). Apoptosis is a highly regulated and controlled cellular process where, by the activation of specific death-signaling pathways, leads to deletion of cells from tissue. These pathways are characterized by profound and

distinct changes in cellular architecture leading to self-destruction, and occurs as part of normal development and aging (Kerr et al., 1994). The distinct morphological features of apoptosis are cell shrinkage, chromatin condensation, membrane blebbing and formation of apoptotic bodies, which are phagocytosed by macrophages without any act of inflammatory response (Jayakiran, 2015).
Physiological cell turnover, shaping tissues during development, and endocrine-dependent atrophy required the apoptosis process (Wyllie, 1997), but also a variety of physiological and patho-physiological stimuli including the damage stimuli from environmental metals (Kerr et al., 1994).
Two major pathways of controlled cell death (Fig.1): mitochondrial pathway (or intrinsic or mitochondria-dependent) and the death receptor pathway (or extrinsic or mitochondria-independent) were already well described (Bratton et al., 1997; Ekert and Vaux, 1997; Ashkenazi and Dixit, 1998; Kaufmann and Hengartner, 2001; Beere, 2004; Gao et al., 2005; Favaloro et al., 2012). In addition to these interrelated pathways, the perforin/granzyme pathway that can induce apoptosis via either granzyme B or granzyme A, was proposed and schematically represented by Elmore (2007) and Taylor et al. (2008) (Fig.1).
The intrinsic pathway is characterized by the permeabilization of the outer mitochondrial membrane and the release of several pro-apoptotic factors into the cytosol, such as cytochrome C, apoptosis induced factor and mitochondrial serine protease (Robertson and Orrenius, 2000; Beere, 2004). Through their ability to regulate mitochondrial cytochrome C release, the Bcl-2 family proteins have a crucial role in the regulation of apoptosis via intrinsic pathway (Bernardi et al., 2001; Taylor et al., 2008). They can be classified into anti-apoptotic (such as Bcl-2 and others), and pro-apoptotic members (such as Bax, Bak, etc.). The *ratio* of the Bcl2/Bax protein could affect the release of mitochondrial cytochrome C (Hildeman et al., 2003). The Bax gene promoter contains a p53-binding site and was shown to be p53 responsive (Benchimol, 2001). The ability of p53 gene to control passage through the cell cycle (in G1 and in G2) and to control apoptosis in response to abnormal proliferative/stress signals, including DNA damage, is considered as fundamental in cell survive (Levine, 1997).
The extrinsic pathway involves the death receptor family which includes receptors that possess a death domain (DD) as FAS, TRAIL-R1 (DR4), TRAIL-R2 (DR5) or TNF-R1 (Jourdan et al., 2009). The trigger component of this pathway is a cascade of cysteinyl aspartic acid-specific proteases known as caspases. The caspases are categorized into upstream (initiating) and downstream (executioner) types. Initiating type (caspase-2, -8, -9, -10 and-12), has being activated by autocatalysis upon sensing death signals; and executioner type (caspase-3, -6, and -7) has being activated proteolysis by upstream caspases (Gao et al., 2005) (Fig.1). A more detailed classification of caspases has been done in mammals into the apoptotic death gene-3 like caspases and the inflammatory interleukin-1β-converting enzyme (ICE)-like caspase (Takle and Andersen, 2007). Caspase-2 is activated by the p53 target gene product.
The unique aspects of fish biology are expressed virtually all the core components equivalent to the mammalian apoptotic machinery (Beere, 2004). In last decades, genetic analysis performed especially on zebrafish; a powerful vertebrate model system, opened new insights to understand apoptosis. Several classes of the genes, the bcl-2 family, Bax, and the caspase family that are homolog to mammalian apoptosis regulators have been recorded in zebrafish DNA databases (Inohara and Nunez, 2000), and most apoptotic pathways are evolutionary conserved between zebrafish and higher vertebrates (Yamashita, 2003).The role of apoptosis on zebrafish development were well characterized (Abraham, 2005). The morphological, biochemical, and physiological information at all stages of early development and in juveniles and adults of both sexes of zebrafish

makes using this species ideal for toxicology research (Hill et al., 2005), including apoptosis occurred as an adverse effects of chemical exposure. In the review of Eimen and Ashkenazi (2010), the various techniques and experimental approaches that are used to study apoptosis in zebrafish were summarized, and the intrinsic and extrinsic pathways comprehensively discussed including developmental apoptosis during embryogenesis, by focusing on the high degree of conservation with humans and other mammals. Therefore, zebrafish could be useful as laboratory experimental animal model in the field of apoptosis to compare with other fish species and mammals.

In zebrafish, it was reported that Mdm2 knockdown embryos were severely apoptotic, and p53 is essential for DNA damage-induced apoptosis during embryonic development (Langheinrich et al., 2002), however, relatively little is known about the specific role of p53 in fish. Although DNA damage (Kawakami et al., 2008) and p53 induction (Ostrakhovitch and Cherian, 2005) were noted as modulators in mammals, a lack of p53 induction was reported in fish cells by model chemotherapeutics (Embry et al., 2006). Apoptosis in zebrafish during early development has beenshown to be associated with activation of p53-dependent pathways (Plaster et al., 2006). Ghiselli (2006) has investigated p53-dependent apoptosis in human and zebrafish cells in relation to the structural maintenance of chromosome 3 (SMC3) protein. SMC3 is a constituent of a number of nuclear multimeric protein complexes that are involved in DNA recombination and repair in addition to chromosomal segregation, and zebrafish SMC3 is 95% identical to the human protein (Ghiselli, 2006).

The extrinsic pathway in zebrafish closely resembles its mammalian counterpart and cooperates with the intrinsic pathway (Beere, 2004; Eimon et al., 2006). Multiple apoptotic components, initiator and effector caspases were described in zebrafish. Moreover, at least six of them have protease domains homologous to mammalian caspase-8 and -10. Several inflammatory interleukin-1β-converting enzyme-like (ICE-like) caspases have also been identified. The catalytic domains of the novel zebrafish caspases, caspy1 and caspy2 share also highest homology with those of human caspase-1 and -5, respectively (Eimon et al., 2006). In addition, the zebrafish genome contains caspase-C and a caspase-C-like protease, which shows highest similarity to human caspase-4. Salmonid genome is also used to classify domain organization of fish caspases. Effector caspases, caspase-3, -6 and -7 (Takle *et al.*, 2006); and a fish homologue of mammalian caspase-14 have been identified in Atlantic salmon, *Salmo salar* (Takle and Andersen, 2007).

In pathway data-base which is a collection of manually drawn pathway representing our knowledge on the molecular interaction and reaction networks for some systems and processes including apoptosis, granzyme-B pathway is also drawn as a major pathway for zebrafish (Kanehisa and Goto, 2000). Granzyme B cleaves its substrates after aspartic acid residues, suggesting that this protease has the ability to activate members of the caspase family directly. The balance between the pro-apoptotic and anti-apoptotic signals eventually determines whether cells will undergo apoptosis, survive or proliferate. TNF family of ligands activates anti-apoptotic or cell-survival signals as well as apoptotic signals. Due to the dynamic and complex nature of apoptotic processes, detecting analyses of controlled cell death by advanced methods such as live imagine were also becoming more attractive in recent years.

Since teleosts are really unique models for studying apoptosis, we previously reviewed the relationships between altering environmental parameters and controlled cell death (AnvariFar et al., 2016, Romano et al., 2013). Surely, the involvements of apoptosis in fish is also be influenced by other various stimuli, especially by environmental chemicals. In parallel with rapid urbanization and industrialization, so many chemicals can enter aquatic system (Scanu et al., 2015), which is

probably assumed as the largest sink. Fishes are very sensitive to aquatic pollutants, and will great promise to make a comparative analysis of environmental chemicals related to toxic stress (Krumschnabel and Podrabsky, 2009). Chemically induced stress can contribute to a variety of pathological conditions and may have a negative impact not only for the fish, but also for consumers of the fish them self (Morcillo et al., 2016). In addition, fish populations inhabiting highly polluted water provide precious information on the etiology of pollutant-mediated diseases such as cancer, and seems to be the practicable tool for health risk analyzes.

Chemical-Induced Stress and Apoptosis

The influence of environmental chemicals on apoptosis can be depended on dose and exposure time. The interactions of environmental pollution and health of fish are always characterized by a vicious cycle: chemicals can lead to stress, and stress seemed to exacerbate the effects of chemicals. As in mammals, immune-neuroendocrine interactions, and the involvements of stress proteins in fish have also a great interest, with particular attention to the modulation of immune responses by antimicrobial proteins (Caccia et al., 2017) and by hormones (Harris and Bird, 2000). The term "stress proteins" is not only used for "Heat Shock Proteins (HSPs)", but also may refer to several other groups of proteins that respond to stressors; MTs, or cytochrome P450 enzymes (Iwama et al., 1998).

HSPs are a suite of highly conserved proteins of varying molecular weight (c. 16–100 kDa) that are produced in all cellular organisms (Roberts et al., 2010), and localized in various intracellular compartments and categorized into families that named as HSP100, HSP90, HSP70, HSP60 and the small HSPs (Lindquist, 1992; Iwama et al., 1998; Morimoto and Santoro, 1998; Feder and Hofmann, 1999; Basu et al., 2002; Roberts et al., 2010). Constitutively expressed HSPs are made up to 5–10% of the total protein content that can be increased two to three times when exposed to stress conditions that cause protein unfolding, misfolding, and a flux of newly-synthesized non-native proteins including the caspases (Pelham, 1986; 1990; Ellis, 1987; Chirico et al., 1988; Deshaies et al., 1988; Lindquist and Craig, 1988; Geething and Sambrook, 1992; Georgopoulos and Welch, 1993;Jolly and Morimoto, 2000; Pockley, 2003; Yamashita et al., 2010; Deane and Woo, 2011). Thus, HSPs can also be defined as key proteins which play important roles in immune reactions, immunomodulation, and apoptosis (Beere, 2004, Romano et al. 2013). Although the majority of studies was focused mainly on the effects of heat shock, some interesting references start to consider HSPs involved in the stress response by high water concentrations of pollutants such as metals and pesticides (Iwama et al., 2004; Mosca et al., 2013). It was suggested that the survival of gray mullet, *Mugil cephalus* under metal-stressed condition may be due to the up-regulation of HSP70, that mediates the altered signal pathway which promotes cellular resistance against apoptosis (Padmini and Tharani, 2014).

Next to HSPs, the metallothioneins, MTs, are also stress-related, low-molecular-weight (approximately 6000-7000 Da), cysteine rich, intracellular proteins that have a great capacity to bind metals. MTs were suggested to act as a cellular-protector against free radicals and other oxidants, or ROS (Vašák and Hasler, 2000). The induction of MTs is often accepted as a sensitive indicator of metal exposition in aquatic animals, despite the fact that MTs induction in fish gills have been revealed to be very species-specific, with some dispute on fish species (De Boeck et al., 2003). As recorded by Wang et al.(2014), MTs not only occur in cytoplasm, but also accumulate in lysosomes, yet could be transported to the nucleus and to the inter-membrane space of mitochondria.

The cause and effect relationships between environmental pollution and controlled cell death are still more attractive. By adding the special information about HSPs and MTs, data on fish apoptosis expands rapidly. In the light of current knowledge, we reported below the effects of some metals and pesticides, BKME, POPs and some other compounds that are familiar or novel, in relation to apoptosis (the synthesis is reported in Table 1).

Metals and Apoptosis in Fish

Heavy metals, semi-metals, transition metals and their compounds naturally enter aquatic environments by various geologic, physical and chemical processes. Surely, anthropogenic activities such as mining, metalworking and industrial processes consistently contribute to environmental concentrations of these compounds. Although some of the metals are essential for living organisms, they becomes toxic when reach to elevated concentrations. The toxicity is closely related to the physical and chemical properties of the metals that are characterized by highly biological activity, bioavailability and ability to bioaccumulation. An overview of references has been reported below about the effects of heavy metals -well known effects of heavy metals and emergent problems related to new types currently highly concentrated in water- in inducing the apoptosis process.

Arsenic (As)

It is a metalloid and naturally exists at high levels in the groundwater of a number of countries, thus enters aquatic environments by various geochemical and anthropogenic processes. As is used industrially as an alloying agent, as well as in the processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives and ammunition. It is also used in the hide tanning process and, to a limited extent, in pesticides, feed additives and pharmaceuticals drink water (Ravenscroft et al., 2009). As noted by Seok et al. (2007), the main source of environmental As exposure is inorganic forms of arsenic (trivalent and pentavalent arsenite), however, organo-arsenic compounds are generally predominat in marine organisms. Its presence in freshwaters has been documented in the past including the possible effect in humans by assuming drink water (Ravenscroft et al., 2009). *In vitro* toxicity of arsenic in fish cell lines is similar to that observed in mammalian cell lines: in grass carp (*C. idellus*) cell culture ZC7901, all of the six heavy metal ions, including pentavalent arsenic, were able to induce apoptosis (Xiang et al., 2001). The liver is one of the most metals-sensitive organ due to the accumulation role. As exposure in zebrafish liver cell line (ZFL cells) has induced a high expression of HSP70; however, a massive apoptosis were observed, indicating that the HSP70 apoptosis-block system has been not able in certain circumstances (Seok et al., 2007). PLHC-1 fish cell line has permitted to reveal that arsenic trioxide-induces necrotic-mediated cell death already at 10 hours while apoptosis has occurred later on, at 40 hours, indicating that the sensitivity of the fish cells to As is also time-dependent (Selvaraj et al., 2013). MTs induction by As are reported in lake whitefish (*Coregonus clupeaformis*) after 64 days of meal supplemented by As (Pedlar et al., 2002). Not surprisingly, the hepatocytes seem to be really sensitive to As since significantly higher rates of apoptosis in hepatocytes were reported in catfish and sea bream species (Roy and Bhattacharya, 2006; Datta et al., 2007; Guardiola et al., 2013). Next to As-induced in liver inflammation and apoptosis, also head kidney and skin seemed to be sensitive tissue to As: it is a directly cause of down-regulation of *mt*, *hsp70* and *hsp90* genes in gilthead seabream skin (Cordero et al., 2014; Benhamed et al., 2016).

The reproductive system of fish seemed to be also high sensitive to As. Japanese eel (*Anguilla japonica*) spermatozoa in *vitro* testicular organ culture system, showed positivity to TUNEL-

reaction that can are enhanced by gonadotropin treatment (Celino et al., 2008), in line also rainbow trout gonad cell line-2(RTG-2) has shown DNA damage after exposure to As (Raisuddin and Jha, 2004). In TO-2 cells (ovary cells of tilapia), treated with sodium arsenite a mitotic arrest was demonstrated (Wang et al., 2004), and concluded that ROS are involved in arsenite-induced apoptosis. After As treatment an intrinsic apoptotic pathway *via* mitochondrial membrane damage was hypothesized in PLHC-1 fish cell line (Selvaraj et al., 2013). Different effects of the As on the immune system were also demonstrated. In catfish exposed to non-lethal arsenic trioxide like the lymphocyte and head kidney-macrophage apoptosis induction (Ghosh *et al.*, 2006; Datta et al., 2009b). Exposure to As can also suppress the overall innate immune function in zebrafish, even if at concentrations deemed safe in drinking water (Nayak et al., 2007). In order to analyze the mechanisms involved in HSP70 induction by arsenic, were used zebrafish liver cell line (ZFL cells), and concluded that oxidative stress induced HSP70 in preventation of apoptosis (Seok et al. (2007). The effects of As are also seemed to be related to apoptosis in developmental stages. Zebrafish embryos (4-120 hours post-fertilization, hpf) threated with high doses of arsenite, exhibited a series of alterations, including genomic DNA methylation disturbances, cell proliferation and apoptosis (Li et al., 2009). As-induced changes in patterns of cell proliferation, cell death, and DNA methylation were also identifiedin developing zebrafish at 24 and/or 48 hpf, by various methods(Lee and Freeman (2014).The As-apoptosis induction was directly linked to a procaspase-mediated mechanism by involving the caspase-3 pathway (Cordero et al., 2014). An induction of ROS production through the activation of NADPH oxidases mediated by caspase-3, was also demonstrated (Datta et al., 2009a; Datta et al., 2009b; Guardiola et al., 2013). However, a reduction in ROS production in zebrafish embryos following *in vivo* exposure to As (Hermann and Kim, 2005) was explained with maturation of the immune system. Banerjee et al.(2011) have evidenced that several signaling pathways including the mitogen activated protein kinase family (MAPK) have been reported to be activated during stress and either promotes or helps in counteracting apoptotic stimuli. Three family members of MAPK, extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (stress-activated protein kinases, JNK) and p38 were studied and concluded that, arsenic-induced alteration in intracellular Ca^{2+} levels initiates pro-apoptotic ERK and inhibitor calpain-2; the two pathways influence each other positively and induce caspase-3 mediated HKM apoptosis.

Copper (Cu)

In fish, Cu is an essential trace metal acting as a co-factor for a number of enzymes, and a structural element of many glycoproteins, so it is needed for erythropoietin processes, antimicrobial peptides formation (Caccia et al., 2017) growth, nervous functions and reproduction. Cu naturally occurs in the aquatic environment in low concentrations. Nonetheless, elevated concentrations originated from agricultural and industrial contamination can be highly toxic to aquatic life. The Cu content seems to concentrated in the basal compartment of the gills (Dang et al., 2000; Monteiro et al., 2012)The exposure in high concentration for short time affect the chloride cells of gills by *via* necrosis, in Mossambique tilapia (*Oreochromis mossambicus*) and Nile tilapia (*Oreochromis niloticus)*, the apoptosis was not revealed probably because the time of exposure has been too short to reveal other typology of damage (Pelgrom ,1995; Bury et al., 1998; Monteiro et al. (2009). In contrast, Xiang et al.(2001) reported that the effects of six heavy metal ions, including Cu, are able to induce apoptosis, in cell culture ZC7901 of grass carp (*Ctenopharyngo donidellus*). In tropical freshwater fish *Prochilodus scrofa*, necrosis and apoptosis of pavement and chloride cells was reported as intense in specimens exposed for long time to high Cu concentrations (Mazon et al.,

2002). The higher incidence of TUNEL-positive cells in gill of the Cu-exposed zebrafish and golden fish, *Carassius auratus gibelio,* were also proved Cu-induced apoptosis (Vergolyas et al., 2010; Luzio et al., 2013). Data on the biochemical mechanism of Cu-induced apoptosis thus is mainly *in vitro*, and suggest that Cu may induce apoptotic cell death via different pathways. Following dietary exposure to Cu, apoptotic cells were only observed at the apex of the intestinal folds of Atlantic salmon, *Salmo* salar (Lundebye et al., 1999). An increase of blast cell apoptosis in hematopoietic tissue during both lethal and sub-lethal Cu exposures was evidenced in *Labeorohita* (Som et al., 2009).The effects of copper exposure has been performed in both larval and adult zebrafish from toxicological point of view, and Hernandez et al.(2011) noted that the most sensitive organs to stress induced by waterborne copper were the central nervous system and the liver, even though the most affected in terms of cell death that is likely to be elicited by the induction of ROS, were the gills and head kidney. Oxidative stress and cell death in lateral line hair cells of zebrafish larvae were induced by acute Cu exposition (Olivari et al., 2008).The apoptotic response of gill to Cu exposure is reported as concentration dependent, with lower concentrations inducing later effects (Luzio et al., 2013). Furthermore, concomitant elucidation of experimental observation of apoptosis was performed by molecular docking with the p53 enzyme with CuO nanoparticles (Kumari et al., 2017). Cell death is likely to be elicited by the induction of ROS, Cortisol- and Cu-induced MT expression in tissues of tilapia was also investigated *in vitro*: when compared to the gills and intestines, the liver evidenced the major synthesis of MT and accumulation of Cu (Wuet al., 2006). The connections of Cu and HSPs are not yet elucidated (Iwama et al., 2004). In PLHC-1 (*Pociliopsis lucida* hepatoma cell line) cells were treated with Cu, a significant induction of both HSP 60 and HSP70 was noted (Moreland et al., 2000). In hepatocytes of rainbow trout, O. mykiss, the Cu-induced apoptosis has been related to increasing amount of HSP70 and lactate dehydrogenase (Feng et al., 2003) and to the upregulation of reactive oxygen species, ROS (Roméo et al., 2000; Krumschnabel et al., 2005; Nawaz et al., 2006), demonstrating a possible independence to caspase-pathway (Li et al., 1998) whereas the latter one pathway might be tissue-specific dependent (Monteiro et al., 2009). More recently, Luzio et al. (2013) have demonstrated that apoptosis was initiated via intrinsic pathway (caspase-9), through p53 activation, then followed by the extrinsic pathway (caspase-8) and finally by the caspase-independent pathway; in the gills of zebrafish exposed to copper. Thus, it is possible to conclude that different apoptotic pathways can be triggered by Cu at different time points.

Mercury (Hg)

Historically, the accumulation of Hg has been revealed in the final predators of the ecological trophic scale and thus in fish, marine mammals and humans, where also toxicological analyses have been focused in these species (rev. Sweet and Zelikoff, 2001). Inorganic and organic Hg cause apoptosis, as has been demonstrated in laboratory by *in vitro* test in zebrafish, sea bass etc. as well as in sample collected *in vivo* in a wide of fish species. In the European sea bass (*Dicentracrchus labrax*), the head-kidney macrophages treated *in vitro* with $HgCl_2$ have been revealed apoptosis as well as the ROS reduced production and the benefits of macrophage-activating factors (MAF) (Sarmento et al., 2004). The most common form of organic mercury is methylmercury (MeHg), that its devastating effect is well documented since Minamata disease, 1960s, where highly toxic chemical bioaccumulated in shellfish and fish in Minamata Bay and the Shiranui Sea, which, when eaten by the local populace, resulted in mercury poisoning, with nervous, skeleton an muscle disease. MeHg accumulation and toxicity were investigated in zebrafish nervous system (Puga et al., 2016) and liver (Ung et al., 2010). Altered expression of clusters of genes involved in apoptosis,

oxidative stress response, transcriptional elongation, or DNA repair were reported in 48-72 hpf zebrafish embryos (Ho et al., 2013). Hg-induced toxicity triggered by oxidative stresses, activate the intrinsic apoptotic pathway, deregulation of nuclear receptor and kinase activities, gluconeogenesis, and adipogenesis. More recently, long established effects of MeHg, such as mitochondrial dysfunction, altered calcium homeostasis, and apoptosis are thought to be consequences of its oxidative stress promoting characteristics (Yadetie et al., 2016). Previous studies that had been performed on the effects of dietary MeHg on gonadal activities of fathead minnows *P. promelas*, walleye, *Sander vitreus*, walking catfish, *Clarias batrachus*, and guppy, *Poecilia reticulate* (Wang *et al.*, 2014), fathead minnow, *Pimephales promelas* (Drevnick et al., 2006). Increased ovarian follicular apoptosis was related to suppressed 17β-estradiol concentrations and smaller ovary size, and it was also suggested that, increased apoptosis as a possible mechanism for the impairment of reproduction in female fish (Homma-Takeda et al., 2001). It was concluded that MeHg may function as an endocrine disruptor by binding to estrogen receptors and acting virtually as an estrogen mimic (Klaper et al., 2006). Data on mercury-induced expressions of HSPs are limited. In subsistence fish, northern pike (*Esoxlucius*), burbot (*Lota lota*), whitefish (*Coregonus nelsoni*), grayling (*Thymallus arcticus*) and sheefish (*Stenodus lencichthys*); no correlation was observed between HSPs expressions in gill and muscle tissues, while HSP 60 or HSP70 protein levels in the gills were correlated (Duffy et al., 1999). The relationships between Hg-induced apoptosis and MTs reported a significant increase dose-related after exposure in the liver of scat *Scatophagus arguss*, (Sinaie et al., 2010), *Liza aurata* (Mieiro et al., 2011) and *C. carpi* (Navarro et al., 2009). However, *Dicentrarchus labrax* revealed a depletion in brain MT content and an incapacity to induce MT synthesis in all the other tissues.

Cadmium (Cd)

Cd pollution originated from industrial effluents is a serious environmental threat in aquatic ecosystem, however, the feeding is still an important route of its toxicity Cd. The acute and chronic toxicity and dangerous bioaccumulation of Cd and consequent apoptosis in fish are documented especially in salmonids. Cultured epidermal cells from explants of skin of rainbow trout exposed to Cd showed typical morphological changes indicative of cell death by apoptosis (Lyons-Alcantaraet al., 1998). Over the skin, the gills are the major route of Cd uptake, as expected the chloride cells are the more sensitive since in fish species as tilapia, high number of immature, necrotic and apoptotic chloride cells and apoptotic bodies were also frequently found (Pratap and Wendelaar Bonga, 1993). In parallel research it has been reported similarly apoptosis in staminal cells of gills and skin after exposure to Cd in carp, *Cyprinus carpio* (Iger et al., 1994) and in *Thalassoma pavo* (Brunelli et al., 2011). The toxicity of Cd to a number of tissues and organs such as liver, kidney and gut was also proved by both of *in vivo* and *in vitro* studies. Cd accumulation in Atlantic salmon was highest in the liver, followed by the intestine and then the gills. Despite the fact that intestinal epithelia provides a much durable barrier than the gill, the rates of apoptosis and cell proliferation in the intestine were more increased by dietary Cd (Lundebye et al., 1999; Berntssen et al., 2001). The exposure to Cd resulted in an increase in apoptotic DNA fragmentation and induced apoptotic cell death in the liver of dab (*Limandalimanda;* Piechotta et al., 1999) and in gut, gills and liver topsmelt(*Atherinopsaffinis*; Rose et al., 2006*)*. Next to this tissues, also the muscles can evidence an accumulation, (in parrotfish, *Oplegnathusfasciatus*; Okorie et al., 2014).

In the ovaries of Cd-exposed Asian cyprinids (*Labeo bata*), lacking of mature oocytes and significantly higher proportion of atretic follicles were noted previously (Das et al., 2005). There was no significant difference in the extent of ovarian follicular cell apoptosis inblack bullhead

(*Ameiurus melas*) and bluegill sunfish (*Lepomis macrochirus*) collected during spring. However, seasonal variation in expression of HSP70, as well as alterations in circulating testosterone levels in female fish chronically exposed to metals were observed (Yoo and Janz, 2003). Expression of genes encoding Zn transporters can be involved in the cadmium-induced reproductive toxicity in female zebrafish (Chouchene et al., 2011). In the testis of *Gobius niger*, based on the increase of caspase-3 gene expression and the presence of its active form in tissue, Cd is concluded a potential apoptotic factor (Migliarini et al., 2005; McClusky, 2006), and MTs protect testis and liver from hazardous effects. In $CdCl_2$-treated zebrafish embryos, Cd-induced apoptosis in the neural tube and defects in axon growth were reported (Chan and Cheng, 2003). Brief $CdCl_2$ exposure causing olfactory cell death during larval development of zebrafish, and the initial wave of cell death occurred immediately following Cd exposure (Blechinger et al., 2007).T ime-dependent apoptotic effects of Cd were also noted in zebrafish, $CdCl_2$, even at a sub-lethal concentration, induces cell death in the brain of embryos but also in adults of zebrafish (Monaco et al., 2017). Possible similarities in the toxicological mechanism of Cd and uranium were also recently noted by Armant et al. (2017), the authors concluded that, cell adhesion and apoptosis are important pathways involved in the mechanisms of toxicity of cadmium, and the detachment of cells from the cellular matrix lead to cell apoptosis, in zebrafish. Apoptosis activation mechanism in fish by Cd has been documented to be of both caspase-dependent and caspase-independent pathways, and even autophagy (Gao et al., 2005). In common carp, Hoole et al.(2003) stated that Cd may mediate their effects on apoptosis via the endocrine system. In cell culture ZC7901 of grass carp, superoxide dismutase (SOD) and catalase (CAT) were also be identified as responsible for Cd-induced apoptotic toxicity (Xiang and Shao, 2003). The apoptosis induced by Cd in primary cultured trout hepatocytes was dependent on the generation of ROS, via mitochondrial cytochrome c (Cyt c) release, and a caspase-dependent pathway (Risso-de Faverney et al., 2001). Confirming this data, Gonzalez et al.(2006) have been evidenced that mRNA levels of apoptosis-related genes (Bax, p53 and c-jun) were up-regulated after exposure to Cd. Although they were confirmed the activation of caspase-3A, and caspase-9, Gao et al., (2013a,2013b) revealed that Cd is not able to activate caspase-8, in purse red carp. It is likely that dose and time dependent actions of Cd-induced apoptosis can occur via different pathways, as observed in arsenic.

Emergent metal contaminants: Aluminum (Al), Chromium (Cr), Silver (Ag), Cobalt(Co) and Zinc(Zn)

Elevated concentrations of aluminum in aquatic ecosystems may originate as a result of erosion and industrial activities, and high concentrations of Al can elicit tissue and organ damages, however, still scarcely investigated in fish. In rainbow trout, *Oncorhynchus mykiss* (Dussault et al., 2001), Al causes damage by swelling and fusion of lamellae, and increased heart rate. Thus, fish death may be caused by iono-regulatory or respiratory failure, or a mixture of both. In Atlantic salmon smolts (*Salmo salar*) the apoptosis of chloride-cells in gills was depended on severity and time-course of Al exposure (Monette and McCormick, 2008); confirmed by acute and subchronic experiment in another species (*Prochilodus lineatus*) where capability of genome repair was observed (Galindo et al., 2010). In mammals, aluminum presumably interacts by binding to either grooves of DNA or the its precipitation (Wu et al., 2005). In fish, the mechanisms were associated with Na/K ATPase, gill mRNA levels of CFTR I (Cystic Fibrosis Transmembrane Conductance Regulator I) and caspase-3B levels (Monette and McCormick, 2008). The cytotoxic and genotoxic effects induced by Al were also determined on common carp erythrocytes, and higher frequencies of micronuclei and

TUNEL-positive cells were observed (García-Medina et al., 2013).. In zebrafish, acute exposure to Al as $AlCl_2$ or Al-nanoparticles cause a decrease in Na1/K1-ATPase activity in gills, presumably resulting in impaired iono-regulatory activity (Griffitt, 2011), however, the authors concluded that nanoparticulate aluminum has little acute toxicity for zebrafish in moderately hard freshwater. Similarly, Kovrižnych et al. (2014) noted that Al nanoparticles are non-toxic for in adulthood and in early life stages of zebrafish. Despite these conclusions, in *P. lineatus*, it seems that the increased catalase and GST activity helped prevent DNA damages caused by Al exposure(Galindo et al., 2010). Furthermore, Al may induce increased lipid peroxidation and protein carbonyl content, as well as unbalance in enzymatic activity that caused an oxidative cell stress(García-Medina et al., 2013; Razo-Estrada et al., 2013).

Al exposure also increases acetyl-cholinesterase activity, and alters behavioral parameters in zebrafish (Senger et al., 2011; Maheswari et al., 2014). Although Al was recently reported as a cardiotoxin in addition to its neurotoxic ability that occured by damaging of astroglial cells- However, no comments was done its apoptotic effects on zebrafish nervous system (Monaco et al., 2017).

While insoluble, trivalent form of chromium (Cr III) is a naturally occurring element, its hexavalent form, Cr VI that spilled from anthropogenic activities has being receive more attention due to its solubility in the last decades. Both forms of chromium are biologically active, as being a strong oxidizing agent, Cr VI can easily across cell membranes. Genotoxic activity of chromium that observed by micronucleus formation, chromosomal aberrations, formation of DNA adducts, and alterations in DNA replication and transcription (Singh et al., 1998). The gill tissues showed more sensitivity as compare with liver in trout (Roberts and Oris, 2004). No data have been reported on other organs or tissue lack the blood. In peripheral erythrocytes of fathead minnow exposed to Cr VI (de Lemos et al., 2001), and *C. punctatus* exposed to Cr III (Choudhary et al., 2012), a significant induction of micronucleated erythrocytes was noted. Cr VI caused to the cytotoxic responses of carp leukocytes. Moreover, changes in shape and reducing amounts of ROS in neutrophils were recorded (Steinhagen et al., 2004). Although appreciable differences in MT induction, SOD activity, lipid peroxidation, cellular morphology, and growth have been reported in rainbow trout (Roberts and Oris, 2004). In common carp leucocytes incubated with Cr VI, Cuesta et al. (2011) noted some changes in shape and reducing amounts of ROS in neutrophils, and depressing proliferation upon mitogen induction, as well as phagocytotic functions, at much lower concentrations that produced cytotoxicity or cell death.

Both *Cobalt (Co) and Zinc (Zn)* are characterized by their enormous applications in industry and mining. Although numerous studies had been performed in different fish species to assess toxicity of Co and Zn, only a few reports were exhibited about Co- and Zn-induced apoptosis. In gudgeon (*Gobio gobio*), roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) were captured from polluted water, good to very good relationships were found between hepatic Zn and hepatic MT levels, however, no data was given in related to apoptosis (Bervoets et al., 2013). In Co-exposed zebrafish embryos, Cai et al. (2012) reported thatthe expression levels of caspase-9, caspase-3 and p53 had been significantly upregulated in a concentration- and stage-dependent manner, and a high level of apoptosis had been detected in the brain, trunk, and tail. Exposure of zebrafish to Co nanoparticles and Co ions caused several gill injuries including necrosis, however, no apoptotic evidence was reported (Mansouri et al., 2015).

Ag Nanoparticles (NPs) are materials of approximately 1-100 nm in length/width, with antibacterial activity (Ju-Nam and Lead, 2008). The AgNPs have able to agglomerate (McShan et al., 2014) and

in this form can provoke apoptosis by several actions: 1) interact with membrane proteins and activate signaling pathways (George et al., 2012; Garcia-Reyero et al., 2015); 2) enter the cell by diffusion/endocytosis to cause mitochondrial dysfunction; 3) could generate ROS; and/or 4) blocks the S phase of the cellular cycle. Interaction of AgNPs with DNA also leads to cell cycle arrest and completely blocks the S phase (Bacchetta et al., 2017).A concentration-dependent increase in mortality and hatching delay, and an increased apoptosis was observed in AgNP treated zebrafish embryos (Asharani et al., 2008). AgNPs exposure can disturb gill and liver function, since a moderate apoptosis was reported. In Japanese medaka, *Oryziaslatipes*, the high level induction of CYP1A that is a ubiquitous member of the P450 superfamily, may contribute to its response to Ag (Chae et al., 2009). It has been demonstrated in zebrafish that oxidative stress, DNA damage, and apoptosis are associated with AgNPs-induced hepatotoxicity by activation ofp53-related pro-apoptotic genes Bax, Noxa, and p21 (Choi and An, 2008). In Japanese medaka, Ag nanospheres-induced chromosomal aberrations and aneuploidy was also observed (Wise et al., 2010). In the liver of the common carp exposed to AgNPs, the upregulation of 502 and down-regulation of 1852 genes were analyzed by using the DNA microarray method (Leeet al., 2012). The differential effects on MT in the liver versus head kidney also suggested potential differences in AgNP deposition/retention in each site since tilapia exposed to AgNP, significantly decreased MT expression levels were observed in liver and spleen, while an increase was noted in the head kidney (Thummabancha et al., 2016).

Bleached Kraft Pulp Mill Effluent (BKME)

As being a complex fibrous mixture of woods, resin, fatty acids, phenols, sterols and terpenes,), Persistent Organic Pollutants (POPs, including the polycyclic aromatic hydrocarbons, PAHs) and heavy metals, called in the complex BKME, produces large volumes of wastewater impacting aquatic environment (Weber and Janz, 2001; Ellis et al., 2004). In perch (*Perca fluviatilis*), profound effects of BKME on several fundamental biochemical and physiological functions, such as very strong induction of certain cytochrome P-450-dependent enzyme activities in the liver, reduced gonad growth and suppression of immune system were reported (Andersson et al., 1988). Reproductive endocrine homeostasis of white sucker (*Catostomus commersoni*) could be altered by BKME chronic exposure, via increasing in ovarian cell apoptosis (Janz et al., 2001). Ellis et al.(2004) has suggested that reproductive endocrine impacts are not due solely to the bleaching process or to chemical additives of pulping, but could also be caused by naturally occurring plant compounds or metabolites. Androgenic effects of BKME was recently reported by observation of gonadal apoptosis (Rutherford, 2011).Although the apoptotic effects of xenoestrogenes is a special issue that not be included to this review, it can be noted that genistein (4′, 5, 7-trihydroxy-isoflavone), one of the endocrine disrupture chemicals of BKME, causes apoptosis through at least two different pathways in zebrafish embryos: (i) it induces apoptosis in an ER-independent manner and (ii) it regulates aromatase-B expression in the brain in an ER-dependent manner (Sassi-Mesai et al., 2009).

POPs include a wide range of halogenated, synthetic, lipophilic or proteophilic chemicals. Their persistence, bio-accumulation, bio-magnification, and long-range transportation are remarkable. Acute, high-level toxicity of POPS is well characterized (UNEP, 2010). 1) Pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene; 2) Industrial

chemicals: hexachlorobenzene, polychlorinated biphenyls (PCBs); and 3) By-products: hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs. However, the list has been expanded formerly to include some polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, and other compounds. Effects of POPs on fish are reviewed recently with a great perspective by Viant et al.(2003). DDTs and dieldrin that mentioned above are known as the most frequently examined POPs,whereas chlordane, PCBs, dibenzodioxins and perfluorinated compounds have been investigated less frequently. Halogenated aromatic hydrocarbons (HAHs) include PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Although using of PCBs has been banned in many countries for several decades, but they are still present in the environment. PCBs occur in 209 different forms, or congeners. Depending on where the chlorine atoms are located, PCBs are classified into two major groups: coplanar (CO) and nonplanar (NP). Coplanar PCBs are considered to be most toxic and referred to as "dioxin-like". Exposure to coplanar congeners of PCB 118 and PCB 77 resulted in an increase in apoptotic DNA fragmentation and induced apoptotic cell death in dab (*L. limanda*) liver tissue (Piechotta et al., 1999). In largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*L. macrochirus*) apoptotic staining in liver, hearth, kidney, intestine and brain were exhibited by PCB exposure (Buckler et al., 2001). More recently, it was reported that chronic exposure to PCBs (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180) could result in hepatic apoptosis in *O. niloticus*. It was revealed a significant activation of caspase 3, and that hepatic caspase (-3, -8, and -9) transcripts were also significantly enhanced (Zheng et al., 2016).Yadetie et al. (2014) demonstrated that genes associated with apoptosis pathways were significantly enriched in juvenile Atlantic cod (*Gadus morhua*) exposed to PCB153. NP PCB153, and CO PCB169 congeners could enhanced levels of ROS in goldfish lymphocytes (Jianying et al., 2009). As expected, coplanar congener was reported more cytotoxic than non-planar one. Aroclor 1254 is a highly chlorinated PCB mixture and its effects in rare minnow (*Gobio cyprisrarus*) larvae has evidenced apoptosis *via* intrinsic and extrinsic pathways (Wu et al., 2014). As noted by Gonzales et al. (2016), relatively few studies have taken advantage of zebrafish model system to examine PCB exposure effects, despite the fact that the developing zebrafish has been used extensively to examine the effects of pesticides and pharmaceuticals on neuro-development and behavior. The authors found that exposure to Aroclor 1254, from 2 to 26 hpf zebrafish embryos induced two statistically significant behavioral defects in larvae at 7 days post-fertilization, but the mechanisms underlying these defects need further studies.

Epigenetic transgenerational apoptotic effects of TCDD was demonstrated in some fish such as medaka(Cantrell et al., 1996, 1998), tilapia (Hart et al., 1999), zebrafish (Dong et al., 2001, 2002) and mummichog, *Fundulus heteroclitus* (Toomey et al., 2001); but not in rainbow trout (Hornung et al., 1999). The mediations of TCDD toxicity was overviewed by Hill et al. (2005).Dong et al (2001) demonstrated increased cell death in the dorsal midbrain of TCDD-treated zebrafish embryos, andnoted that incidence of apoptosis as inversely related to blood flow in this brain region following graded TCDD exposure concentrations (Dong et al, 2002). TCDD-induced apoptosis was also characterized in medaka, DNA degradation in cells of the embryonic vasculature and loss of functional integrity of the medial yolk vein of TCDD-exposed embryos were demonstrated (Cantrell et al., 1996, 1998). The authors suggested the relationships of the expressions of cytochrome P450 1A and cytochrome P450, while CYP1A expression was proposed by Toomey et al. (2001). The aryl hydrocarbon receptor (AHR) pathway was also recommended for not only TCDD, but also polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans

(PCDFs) and coplanar PCBs (Dong et al., 2001; 2002; Karchner et al., 2005; King-Heiden et al., 2012). AHR is a ligand-inducible transcription factor, as a member of the basic helix-loop-helix/Per-Arnt-Sim family of proteins, and well known for mediating the toxic effects of TCDD. In addition to two AHR genes, AHR1 and AHR2 that was previously identified in zebrafish, a new zebrafish AHR, designated as AHR1B was reported by Karchner et al. (2005). Zebrafish AHR2 binds TCDD with high affinity, is transcriptionally active and has a major role in mediating the developmental toxicity of TCDD. Zebrafish AHR1 lacks the ability to bind TCDD and activate transcription, and currently with unknown function. The authors speculated that AHR1B may have a physiological role, such as in embryonic development, whereas AHR2 mediates the response to xenobiotics.More recently, in order to develop a convenient and sensitive biomonitoring tool to examine the level of POPs in the environment and evaluate its potential human health risks by TCDD, Luo et al. (2018) established a transgenic zebrafish model with a red fluorescent reporter gene (mCherry) using the truncated cyp1a promoter.

Perfluorinated organic compounds (PFCs) are emerging POPs (Stahl et al., 2014). Perfluorooctanoic acid (PFOA) is one of the most used PFCs and revealed oxidative stress and apoptosis in primary cultured hepatocytes of tilapia (Liu et al., 2007). In zebrafish, general mode of action of PFOA was described as an increase of the mitochondrial permeability and cell death (Hagenaars et al., 2013). Perfluorooctane sulfonate (PFOS) is a persistent organic pollutant and causes oxidative stress, apoptosis, and developmental toxicity in zebrafish embryos. Shi and Zhou (2010) stated that, in PFOS- exposed 4 hpf zebrafish embryos, extracellular signal–regulated protein kinase (ERK) expression levels were unchanged, whereas terminal kinase (JNK) and p38 gene expressions were significantly upregulated, which could be linked to PFOS-induced cell apoptosis in zebrafish larvae. Du et al. (2017) revealed that PFOS plus ZnO-NPs co-exposure could cause more serious oxidative stress and apoptosis. The expressions of Bax, p53, caspase-3 and caspase-9 were significantly up-regulated in the PFOS plus ZnO-NPs exposed zebrafish embryos, while anti-apoptotic gene Bcl-2 was significantly down-regulated. Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are organo-bromine compounds that are widely used as flame retardant in a wide array of products (Covaci et al., 2006), and added to POPs list in 2009 and 2013, respectively. Deng et al.(2009) stated that HBCD can induce oxidative stress and apoptosis in zebrafish by down-regulation of anti-apoptotic genes. PDBE 47 and PDBE 99 expositions of thymocyte culture of lake trout, *Salvelinusnamaycush* caused apoptosis, and necrosis (Birchmeier et al., 2005). In zebrafish, Usenko et al. (2012) reported that hydroxylated PBDEs disrupt development, and may induce oxidative stress and potentially disrupt the cholinergic system and thyroid hormone homeostasis. However, the authors had not observed apoptosis by using caspase-3 assay. Exposition to PDBE metabolite-OH-BDE-47, resulted in significantly increase of apoptotic cells in the brain of zebrafish embryos from 4 hpf until 22, 26, 30, 34, or 96 hpf (Wang et al., 2018). Hexachlorocyclohexane (HCH) is any of several polyhalogenated organic compounds that have many of isomers using as insecticides. In lake troutexposed to Aroclor 1254 and alpha, beta, gamma, delta isomers of HCH, an apoptotic effect on thymocyteswas reported (Sweet et al., 1998). Although it was recorded that HCH have serious effects on ovulation and fertilization in female zebrafish, (Yüksel et al, 2016), no data was found on its apoptotic effects. As expected, QSAR (quantitative structure–activity relationships) parameters can be versatile tools for prediction of the toxic effects of chemicals. In order to elucidate the *in vitro* apoptotic cytotoxicities of POPs on *C. auratus*lymphocytes, Zhang et al.(2008a)used some physicochemical parameters of 25 different chemicals including chlorobenzenes (CBs), PCBs, and DDTs. The apoptotic effects of all of the

substituted aromatic chemicals were confirmed by DNA ladder and nucleus condensation. The apoptotic EC_{50} data were best correlated with the dipole moment and the energy of the lowest unoccupied molecular orbital.

Polycyclic aromatic hydrocarbons, PAHs are natural and/or anthropogenic derivatives and the most potently carcinogenic among POPs. They are benzo-a-pyrene (BaP),7,12- dimethylbenz-a-anthracene (DMBA), and 3-methylcholanthrene (3-MC). In *Fundulusheteroclitus* fry acutely exposed to dimethyl sulfoxide (DMSO) control, BaP, or DMBA, bile duct epithelial cells (cholangiocytes) apoptosis and differentiation were noted as related to CYP1C1 expression (Wang et al., 2010).Weber and Janz (2001) exposing juvenile channel catfish (*Ictalurus punctatus*) acutely to the AHR agonists, β-naphthoflavone, or the model PAH, dimethylbenz-a-anthracene (DMBA) via intraperitoneal injection, have noted a significant negative relationship between expression of HSP70 and apoptosis. In eel, *Anguilla anguilla* exposed to BaP, Aroclor 1254, 2-3-7-8-tetrachlorodibenzo-p-dioxin and β-naphthoflavone, evidenced a significant induction of apoptosis (Nigro et al., 2002). 3-MC, that can be found environmentally, induced apoptosis in both lymphocytes and phagocytes of common carp, in dose dependent manner (Reynaud et al., 2004). Reynaud and Deschaux (2006) evidenced that 3-MC induced-apoptosis in carp was calcium dependent in both lymphocytes and phagocytes. Bakhtyar and Gagnon (2011) were investigated the effects of BaP on the Australian native fish pink snapper (*Pagrus auratus*), mulloway (*Argyrosomus hololepidotus*) and barramundi (*Lates calcarifer*). For all species, levels of HSP 70 measured in the gills remained unchanged following the treatment with BaP, however, DNA integrity was affected in all three species of fish, and the appearing of fragmented-apoptotic cells in peripheral blood and head kidney after the exposure to nonylphenol and to spiked oil was reported. In 30 hpf zebrafish embryos exposed to different concentrations of BaP, some morphological abnormalities such as pericardial edema, reducing in eye and jaw growths were revealed, however, no conclusion was done on possible relationships between apoptosis and abnormalities (Kim et al., 2014). It was noted that decrease in ovarian HSP70 expression in response to DMBA (Weber and Janz, 2001; Baršienė et al., 2006). The high concentration of BaP impaired tilapia immune function and can induce apoptosis in the head kidney (Holladay et al., 1998). Fluorine is a member of the halogen family widely distributed in the environment. Cao et al. (2013) stated fluoride caused oxidative stress and a dose-dependent apoptosis was induced in the common carp. Comparative proteomic analysis of the kidney, liver, and cardiac muscle samples from puffer fish *Takifugu rubripes* exposed to excessive fluoride has revealed five or more proteins, up-regulated or down-regulated, seem to be involved in apoptosis and other functions associated with fluorosis (Lu et al., 2009, 2010a, 2010b). Tributyltin (TBT) compounds are an antifouling agents for ship-bottom paint. Reader et al.(1999) evidenced TBT triggered apoptosis through increase in intracellular Ca^{2+} levels also activation of calpain-like proteases which down-regulated protein kinase C (PKC), that this down-regulation or degradation of PKC, a key event in TBT-induced apoptosis could modify the phosphorylation status of Bcl-2 homologues and lead to apoptosis in trout hepatocytes. Although TBT has seemed to affect the reproductive system of fish, the effects on gonadal development and follicle maturation remain unclear. However, a significant increase in apoptotic ovarian follicular cells of TBT-exposed in *Sebastiscus marmoratus* has been noted (Zhang et al., 2007). The trimethyltin (TMT) is a short-chain trialkytin used in industry and agriculture (Gomez et al., 2007).Wang et al.(2008) stated TMT induced apoptosis in brain cell by increased production of ROS, nitric oxide (NO) and caspase-3 of false kelpfish. In 48 to 72 hpf zebrafish embryos, exposed to TMT has been observed significantly apoptosis, revealing a strong effect also in fish

development (Chen et al., 2011). Nonylphenolethoxylate is present as pollutant in the aquatic environment, it breaks down to 4- nonylphenol (NP) which is known more stable and persistent. The high concentrations of NP caused apoptosis in blood cells of *Clarias batrachus* (Ateeq et al., 2002). Mekkawy et al. (2011) have recorded apoptotic erythrocytes with many malformations in *C. geriepinus* exposed to sublethal concentrations of NP. When compared to controls, a six-fold greater extent of apoptosis in spermatocytes, Sertoli cells and Leydig-homologue cells, but not in spermatids of testes from NP-exposed male medaka, as was reported by Weber et al.(2002). 4-Nonylphenol has induced apoptosis in the notochord, trunk, medial fin and in the brain of 33 hpf zebrafish embryos, and it has suggested that might be due to cellular damage caused by oxidative stress (Chandrasekar et al., 2011).

Pesticides

Due to their widespread using, persistence and capability of bioaccumulation, agrochemicals are very hazardous to the aquatic environment. The effects of insecticides on fishes are of great concern, and it is well known that zebrafish is an unique tool to assess the apoptotic effects of pesticides during developmental stage. For example, exposure to atrazine, 2,4-D, DDT, dieldrin, and malathion cause apoptotic cell death in the brain region of 96 hpf zebrafish embryos (Ton et al., 2006). Insecticides are generally accepted as the most acutely toxic class of pesticides, therefore, in this part of this review we will talk about six groups of insecticides classified as organophosphorus compounds (OP), organochlorin compounds (OC), carbamates (CB), pyrethroids, neonicotinoids and botanical pesticides.

Organophosphorus Pesticides (OPs)

OPs that derive from the phosphoric or phosphorothioic acid (Roberts and Reigart, 2013), and their effects on fish have been recognized and reviewed unless limited data are available regarding apoptosis effect. OPs exhibit their toxicity primarily by phosphorylation of the acetylcholinesterase (AChE). Moreover, OPs induced oxidative stress can trigger the cell death (Shen and Liu, 2006). Lipid peroxidation resulting from ROS can be easily detected by increasing of malondialdehyte (MDA) concentrations. It is also known that the involvement of JNK in controlling diverse cellular functions such as cell proliferation, differentiation, and apoptosis is based on phosphorylation and functional modification of these molecular targets in stimuli-and cell-type-dependent manners (Shen and Liu, 2006). OPs are also known to cause immunosuppression, although there have been only few studies involving fish, and the immunotoxic mechanisms are not clear.Díaz-Resendiz et al.(2016) stated that, late apoptosis and loss of mitochondrial membrane potential in lymphocytes of Nile tilapia were provoked by diazinon. Although their effects on gills, liver, hematological parameters, ovary and testis are well documented, no reports were available on fenthion- and fonofos-induced apoptosis. By employing multiple qualitative and quantitative methods, it has been shown that exposure of cell line ZC-7901 of grass carp fish to malathion induces a decrease in the mitochondrial membrane potential and apoptosis via a direct effect on the mitochondria (Chen et al., 2006). The induction of lipid peroxidation that resulted with ROS generation by methyl parathion was reported in gills, liver and brain of guppy, *Poecilia reticulate* (Sharbidre et al., 2011). Apoptotic effects and changes in glucose-6-phosphate dehydrogenase (G6PD) enzyme activity in liver and gill tissues of rainbow trout exposed to chlorpyrifos were investigated, evidenced apoptosis in gill and liver by inhibiting of G6PD (Topal et al., 2014). In Atlantic salmon (*S. salar*) liver cells, the antioxidant, vitamin E had only a modest effect on chlorpyrifos-induced oxidative

damage leading to apoptosis (Olsvik et al., 2015). Chlorpyrifos exposure were caused to controlled cell death and disturbed mRNA expression levels of c-myc, cyclin D1, Bax and Bcl-2, in zebrafish embryos (Yu et al., 2015). An activation of cell apoptosis in the brain of both embryos and larvae of zebrafish has been reported after an exposure to methamidophos (He at al., 2017) indicating also an effect on nervous system in vetebrates.

Organo chlorinated Pesticides

This group of pesticides comprises many of the most toxic and persistent compounds for aquatic environments, such as DDT and relatives, dieldrin, lindane, chlordane, and endosulfan. p,p′-DDE evidenced increase apoptosis on the immune system of Chinook salmon *Oncorhynchus tshawytscha* by a reduction in lymphocyte-granulocyte viability detected by increasing the percentage of apoptotic cells in spleen and head-kidney of juvenile Chinook salmon (Arkoosh et al., 1998; Misumi et al., 2005). Contrary, Cuesta et al. (2008) have observed no changes in innate cellular immune parameters in marine gilthead seabream leucocytes. Testicular apoptosis in *Clariasgari epinus* and *O. mossambicus* inhabiting a DDT sprayed area were demonstrated in detail by Patrick (2008). Interestingly, no strong positive or negative correlation between the increased number of apoptotic cells was revealed. Dieldrin induces neurotoxicity in the vertebrate central nervous system (CNS) including teleostean and impairs reproductive processes in fish (Ton et al., 2006). Dieldrin exposure can cause prolonged oxidative stress, and may result in mitochondrial dysfunction and the release of cytochrome C into the cytosol, leading to apoptosis (Kitazawa et al., 2003). Lindane (gamma-hexachlorocyclohexane) has provoked in gilthead seabream an impariment of the immune functions with head kidney leucocytes decrease (Cuesta et al., 2008). Although it was revealed that chlordane can accumulate in fish tissue, endosulfan is a potent OC pesticide from the cyclodiene group and is widely used and Tellez-Bañuelos et al.(2011) have studied the in *vitro* effects of this insecticide in Nile and could in turn it have an anti-apoptotic effect.

Carbamates (CB)

It is believed that CB, like OPs, show their effect through inhibition of AChE, or/and butyryl-cholinesterase (BChE), as well as disturbing the metabolism of other neurotransmitters such as gamma-aminobutyrate (GABA). As being a member of carbamates, carbaryl is also an AChE inhibitor that prevents the breakdown of acetylcholine, retards embryonic development, affects embryo size, and delays hatching. In carbaryl-treated zebrafish embryos, the amount of cell death that was experienced exceeded that of controls. TUNEL assays suggest that cell death in heart cavity and the extent of the brain and spinal cord may be due to apoptosis and is consistent with cardiac and neuronal defects (Schock et al., 2012). Carbendazim, a carbamate ester-amine that is widely used as fungicide, was previously reported as harmful to Prussian carp embryonic development and hatching (Ludwikowska et al., 2013). It has been recently shown that carbendazim has potentiality to induce cell apoptosis and can causes immune toxicity and endocrine disruption in zebrafish embryos (Jiang et al., 2014). Upon the exposure to the same concentrations of carbendazim, the expression patterns of many key genes involved in cell apoptosis pathway (e.g. p53, Mdm2, Bbc3 and caspase-8) were significantly up-regulated, while the Bcl2 and caspase-3 were down-regulated.

Pyrethroids

These synthetic chemicals that share some similarities with natural pyrethrins, are known as the safest insecticides that used worldwide, but they are highly toxic to most of fish in time-dose dependence. Cypermethrin (CYP) cause hepatic DNA damage and up-regulate genes related to apoptosis in the liver and nervous system in zebrafish embryos (Shi et al., 2011; Jin et al., 2011a).

In zebrafish, several apoptosis-related genes, such as p53, Apaf1 and Cas3, were significantly upregulated after CYP exposure, while Bcl2/Bax expression ratio had been decreased (Jin at al., 2011b). In chronically exposed to deltamethrin, early apoptotic signs on the gills of carp (*C. carpio;* Cengiz, 2006) and ovarian cells of Nile tilapia have been observed (*O. niloticus;* Calma et al., 2004). Up-regulation of chaperons stress genes, down- or non-regulated cytokines in Chinook salmon, *O. tshawytscha* (Eder et al., 2009). Fenvalerate exposure resulted in apoptosis in the brain of zebrafish embryos and larvae (Gu et al., 2010).In delta smelt (*Hypomesus transpacificus*) exposed to esfenvalerate, alterations in the expression of genes associated with immune responses, along with apoptosis, were reported (Connon et al., 2009). The exposure to this substance has been also caused alterations in immune responses, along with apoptosis of leukocytes in lymphomyeloid organs (Kaviraj and Gupta, 2014). Piperonylbutoxide that used for prolong the effects of many synthetic insecticides, including phyrethroids, was reported as a modulator caused to increase the oxidative stress potential and apoptotic effects of lambda-cyhalothrin, in the liver of *O. niloticus* (Piner and Üner, 2012).

Neonicotinoids

The growing use of neonicotinoids, the new classes of insecticides, which degrade rapidly in water have a great concern in last decades. Due to their growing use, imidacloprid, clothianidin, dinotefuran, thiacloprid and acetamiprid, have raised a great concern in last decades. Neonicotinoids are distributed systemically throughout the growing plant following seed or soil applications, and another recent insecticide, fipronil, a phenyl-pyrazole (fiprole) rather than a neonicotinoid, has a similar toxicity and persistence profile. In fish, neonicotinoids could act by binding to nicotinic acetylcholine receptor (nAChR) in the postsynaptic neuron (Gibbons et al., 2015). *In vitro* acute cytotoxicity in gill cell line of flounder, *Paralichthy olivaceus* after exposed to neonicotinoid insecticide imidacloprid evidenced a possible intrinsic pathway of apoptosis induction (Su et al., 2007).

Biopesticides

The term “biopesticides” is defined by EPA as naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs. Natural pesticides can also be toxic, askethrin (obtained from roots of *S. flavescens*) that evidenced toxicity for the fresh water fish *Labeorohita* (Bhatt and Nagda, 2012). Camptothecin (CPT), isolated from the bark and stem of tree *C. acuminate*, is a cytotoxic quinoline alkaloid, beacause block DNA-repairing enzymes and thus apoptosis bodies (Venditto and Simanek, 2010), and in silver sea bream, *Sparussarba* determined DNA fragmentation and apoptosis (Deane and Woo, 2005). CPT was recorded as being a topoisomerase 1 inhibitor, in zebrafish (Kari et al., 2007) causing deformed embryos and some tissue, like muscle, peripheral nerve and bone have shown apoptosis (Ishaq et al., 2017). Azetidine-2-carboxylic acid (Aze) accumulates notably in the bulbous roots *B.vulgaris*, could interfere in the capacity of HSP70 synthesis in different fish species-cell line in combination with cortisol (Deane et al., 2006).

Conclusion

Fish are ideal organisms to assess and solve environmental problem since they are favorable bioindicators with their high sensitivity to toxicants. In this view, this review could be a comprehensive panorama of the knowing cell-toxicity to environmental pollutants. However, it was

removed from this manuscript the part relative to toxicology from bacteria, algae and protozoan that can occur in pollutants waters, because needs a deep and long-write discussion. Therefore we have decided to treat this precise topic in a future review. At this point, we have also to note that chemical-induced apoptosis in the manner of hormonal interactions is purposely outside the scope of this review. Along the manuscript, the zebrafish has revealed to be a fish model-species, due to be better suited to answer some special questions on apoptosis. Zebrafish has a well-known genotype, and a very sensitive freshwater species. The capacity to survive to chemical pollutants of zebrafish by AMPs expression (Caccia et al. 2017) or by the other cellular and extracellular molecules (Sales et al., 2017) open a new approach to translational studies in future for immune disorders and cancer (Moiseenko, 2010). For example, using of a liposomal formulation of hepcidin, AMP from tilapia, and epirubicin, an anti-neoplastic agent, can lead to cell death in human squamous carcinoma and pluripotent testicular embryonic carcinoma by activation of intrinsic pathway (Lo et al., 2015).

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