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Alterations in viability and *CYP1A1* expression in SH SY5Y cell line by pollutants present in Madín Dam, Mexico

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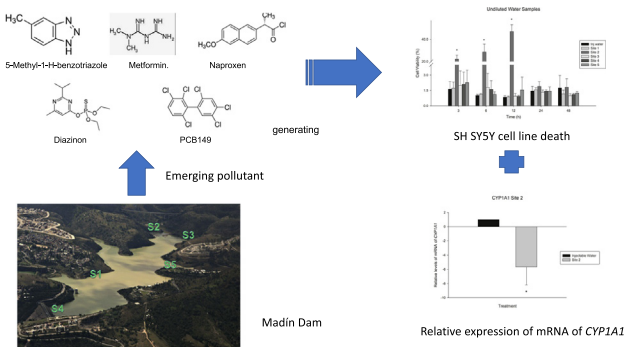
HIGHLIGHTS

- Pollutants and concentration at each site modifies the human cellular response.
- Potential protective effect of contaminant can enhance cell survival.
- The toxic effects of pollutants are time dependent.
- Madín Dam contaminants modify the normal expression of *CYP1A1*.
- Alterations of *CYP1A1* expression could be related to neurodegenerative diseases.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently one of the problems facing global development is the availability of water. Although water is abundant the planet only a small portion is for human use and consumption. The problem is exacerbated due to different factors, mainly: meteorological phenomena, the presence of contaminants in the water and the increase in the number of inhabitants. Potential effects of pollutants not only can affect freshwater biota but also can be implicated in cancer development and neurodegenerative diseases in humans. The study was conducted in the Madín Dam, a reservoir of economic importance for the geographical area in which it is located, as well as catering to the population of nearby areas, and is a place where recreational activities such as fishing and kayaking are carried out. The aim of this study was to identify the toxic effects that the pollutants present in the water of the Madín Dam can generate on a human cell line (SH SY5Y) evaluating the cell viability and the participation of the Aril Hydrocarbon Receptor (AhR) and Pregnane X receptor (PXR) through of the expression of the *CYP1A1* and *CYP3A4* (canonical genes). In one of the five sites analyzed, cell viability was up to 50%, in this site a decrease in the normal expression of CYP1A1 was observed ($p < 0.05$) and the CYP3A4 gene was not expressed in the cells SH SY5Y. These results show that the SH SY5Y cell line is a good biomarker for assessing the human toxicity of environmental pollutants and relating it to neurodegenerative diseases.

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1. Introduction

The water resources accessible for human use have their origin in rainfall (Schwan et al., 2019). Before the hydrological balance was altered to meet human needs, runoff sustained all ecosystems (NOM-011-CONAGUA-2015, n.d.). Until the 19th century, the gradual reduction of natural runoff due to the growth of anthropogenic activities did not cause serious damage to the environment. However, in the course of the twentieth century, the use of water to satisfy domestic activities, for food production (agricultural and livestock use) and for industrial activities increased rapidly (Escalas et al., 2019; Mititelu-Ionuş et al., 2019). These facts became more evident during the second half of the twentieth century, which generated serious and in some cases irreparable damage to ecosystems (Ahkola et al., 2017). Several reports published by the World Health Organization and other agencies interested in the environment present alarming data on water availability and affordability (Bhati and Rai, 2017). It is estimated that between 17 and 19 million people in the world lack access to drinking water (Amiri et al., 2019).

In Mexico, groundwater is a vital resource for the development of all sectors including agriculture, livestock, industrial and commercial (NOM-014-CONAGUA-2003, 2008; Dehghani et al., 2019). Considering that in >50% of the national territory dry and semi-dry climates prevail, the large reserve of groundwater in regional aquifers is a valuable resource that has led to the development of arid areas (NOM-014-CONAGUA-2003, 2008). In the 60s to 80s the demand for groundwater increased considerably, with a slow renewal of this resource (Gavrilescu et al., 2015). In addition, water pollution caused by industrial, hospital and domestic discharges has aggravated the problem (Kaur et al., 2019; Subbiah et al., 2019; Xu et al., 2019; Yang et al., 2019).

Anthropogenic activities combined with some environmental disasters (such as floods, forest fires, volcanic eruptions) have favored that water bodies on the planet are impacted with various pollutants (Crawford and Quinn, 2017; Escalas et al., 2019; Hu et al., 2019; Shao et al., 2019; Windsor et al., 2019). Some of them are persistent substances, heavy metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), solvents, pharmaceuticals, pesticides, personal care products and microcontaminants (Chen and Liu, 2019; Da et al., 2019). Many times the concentrations of these are very low, which makes it difficult to assess their presence, fate and deleterious effects on ecosystems and hydrobionts (Kaur et al., 2019; Windsor et al., 2019). The main problem of the presence of pollutants in mixtures are interactions, which can generate synergistic or potentiating effects, affecting organisms or the ecological balance of ecosystems (Dehghani et al., 2019; Jiang et al., 2019).

Dams in Mexico and the world are important as they supply drinking water to the surrounding municipalities and because these sites can develop recreational activities such as sailing, fishing and kayaking. In the State of Mexico, contaminated water bodies have been identified, such as the Madín Dam, a reservoir fed by the Tlalnepantla River, this dam is located between the municipalities of Naucalpan de Juárez, Atizapán de Zaragoza and Tlalnepantla de Baz. These municipalities are important since they are part of the suburbs of Mexico City. The main source of contamination for this dam comes from domestic discharges from human settlements and from industrial waste and nearby businesses (Galar-Martínez et al., 2010; González-González et al., 2014; Morachis-Valdez et al., 2015; Pérez-Coyotl et al., 2017, 2019).

Previous studies such as González-González et al. (2014) show that the pollutants present in the Madín dam (metals and nonsteroidal anti-inflammatory drugs, mainly) induce oxidative stress in the blood, gills and muscle of *Cyprinus carpio* (common carp) cataloged as sentinel organism. Also, Morachis-Valdez et al. (2015) identified changes in the physicochemical and textural properties in muscle of *C. carpio* by exposure to the same contaminants.

Studies conducted by Pérez-Coyotl et al. (2017) showed that the contaminants present in the water of Madín dam (MD) also generate oxidative stress, genotoxicity and cytotoxicity in *C. carpio.* Also, Pérez-Coyotl et al. (2019) demonstrated that the effluents of this dam cause embryolethality, embryotoxicity, congenital anomalies and oxidative stress in common carp embryos. These studies, together with others carried out worldwide, have shown that the waterbodies are contaminated with various substances and that many of these exceed the permissible levels for aquatic life and, consequently, may have effects on human health (Ahkola et al., 2017; Blair et al., 2019; Da et al., 2019; Jiang et al., 2019; Kaur et al., 2019; Mititelu-Ionuş et al., 2019).

Prolonged exposure even to low concentrations of pollutants present in the environment can have subtle but significant effects on human health, for example, the interaction of neurotoxic chemicals with the normal aging process can be very slow and progressive and difficult to detect (Baltazar et al., 2014; Bondy, 2016; Crawford and Quinn, 2017). Pharmaceutical and personal care products have been identified as endocrine disruptors causing serious health problems in the population, in addition to the development of resistance in microorganisms (Ahmed et al., 2017; Elizalde-Velázquez et al., 2016, 2017; Orozco-Hernández et al., 2018, 2019). In the case of organophosphates and polychlorinated biphenyls, they have been identified as probable human carcinogens, potent suppressors of the immune system, causing oxidative stress, neurotoxicity, hepatotoxicity and incidence of neurodegenerative diseases (Baltazar et al., 2014; Gräns et al., 2015; Bondy, 2016; Ash et al., 2017; Crawford and Quinn, 2017; Rodriguez et al., 2018; Osawa et al., 2019; Roy et al., 2019).

The toxicity of dioxin-like compounds and the induction of *CYP1A* gens are mediated by activation of the aryl hydrocarbon receptor (AhR). AhR is a member of the helix-loop-helix/per-Arnt-Sim (bHLH/PAS) transcription factor family that is activated by natural and synthetic ligands such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and other environmental pollutants (Calò et al., 2014). AhR is constitutively expressed, but after ligand binding, it is translocated from the cytosol to the nucleus where it forms a heterodimer with the nuclear translocator of the aryl hydrocarbon receptor (ARNT) and binds to the xenobiotic response elements (XRE) by regulating the expression of a battery of genes encoding xenobiotic metabolizing enzymes, such as cytochrome P450 (CYP1A1, CYP1A2, CYP1B1), in addition this factor has important functions in liver and cardiac development, cell proliferation, cholesterol and glucose metabolism, the circadian cycle, the ubiquitin proteosome system, homeostasis and the immune response (Mejia-Garcia et al., 2013; Roy et al., 2019).

In addition to AhR, another receptor involved in the biotransformation of drugs is the pregnan X receptor (PXR). PXR is expressed in the liver, intestine, mainly and to a lesser extent in the lungs, stomach, adrenal gland and some regions of the brain. This has a fundamental role in energy homeostasis, inflammatory response in liver and extrahepatic tissues due to xenobiotics (Istrate et al., 2010; Pavek, 2016; Yoshinari, 2019).

As mentioned earlier, the contamination of water bodies has serious consequences on the environment, where various study groups have focused their experiments on sentinel organisms (Kaur et al., 2019), but it is very important to evaluate the quality of water bodies as well as possible effects that could favor the incidence of diseases in humans, particularly neurodegenerative diseases (Ball et al., 2019), in vitro studies are required and cell lines can be widely used as in the case of the SH SY5Y cell line in Parkinson studies but also they are used in studies related to diseases such as Alzheimer, ischemia and amyotrophic lateral sclerosis (Xicoy et al., 2017). This line is a subline of the SK-N-SH line, which was established in culture in 1970 from a bone marrow biopsy of a metastatic neuroblastoma of a 4 year old patient (Kasemeier-Kulesa et al., 2018).

The aim of this study was to evaluate the human toxicity of the contaminants present in the water of the Madín dam through viability and the expression of the *CYP1A1 and CYP3A4* gene in the SH SY5Y cell line, which will help establish the possible association between pollutants and harmful effects on human beings, which will further favor the

development of pollutant management and regulation programs that reduce the environmental and population damage evidenced in recent decades (Gómez-Gutiérrez et al., 2016; Blair et al., 2019).

2. Methods

2.1. The study area and water sampling

The MD (at 19°31′37″N and 99°15′33″W) has a surface area of 190 ha and a storage capacity of 16.6 million m^3. It is bordered by the towns of Nuevo Madín and Viejo Madín, which discharge their waste directly into the reservoir. The dam was built to control the flow of the Tlalnepantla river and provide drinking water to nearby municipalities.

Water samples were obtained from 5 sampling sites at MD, State of Mexico. Site 1 = Discharge Nuevo Madín (S1); Site 2 = Entrance of the tributary of the Tlalnepantla River (S2); Site 3 = Side branch of the reservoir (S3); Site 4 = Curtain of the dam (S4); and Site 5 = Discharge of Old Madin (S5) (Fig. 1).

Water samples from the sampling sites were collected twenty-liter polyethylene containers in the month of September 2016, during the rainy season. The sampling was performed according to the Mexican norm (NMX-AA-003-1980). The containers where the water of each site was collected were previously washed with 30% nitric acid and rinsed perfectly with deionized water. Once the samples were obtained, they were identified and protected from light and transported to the laboratory. They were stored at 4 °C, prior to analysis.

The sampling sites used for this study were previously evaluated by our research group, mainly detecting embryotoxicity, genotoxicity and cytotoxicity, this evaluated thorough the use of sentinel species. The concentrations of pollutants present in each of the MD sites were obtained from the data reported by Pérez-Coyotl et al. (2019). Overall, 73 microcontaminants were identified in the five sampling sites belonging to the groups of personal care products, pharmaceuticals, pesticides, and persistent organic pollutants. Table 1 summarizes the most representative pollutants of each group. The site 2 has high concentrations of contaminants for all identified groups, except for the personal care products group. Also, in the toxicity studies conducted in MD it has been one of the sites that presents the highest toxicity in *Cyprinus carpio* in responses such as oxidative stress, genotoxicity and embryotoxicity (González-González et al., 2014; Pérez-Coyotl et al., 2017, 2019).

2.2. Cell culture

SH SY5Y cells were obtained from the American Type Culture Collection (Manassas, VA, USA), and were grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% bovine fetal serum (HyClone, Logan, UT, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37 °C with 5% CO_2.

Fig. 1. Sampling sites in Madín Dam. S1. Download Nuevo Madín, S2. Point of entry of the tributary of the Tlalnepantla River, S3. Side branch of the reservoir, S4. Curtain of the dam, S5. Old Madín download.

2.3. Evaluation of cell viability

Cell viability is the proportion of specific living and functional cells in a culture. It serves as a quality control and represents a predictive biomarker to assess toxicity. Specifically, the SH SY5Y cell line is a good predictor of neurotoxicity due to its similarity with neuronal activity.

Cell viability was assessed with a rapid colorimetric assay, based on the reduction of MTT tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma-Aldrich). This method is generally used to measure cytotoxicity, proliferation or activation with a high degree of precision. The metabolic activity of the cells on MTT includes the mitochondrial dehydrogenases, in particular succinate dehydrogenase, therefore the test has a high degree of sensitivity.

To assess cell viability, 25,000 cells were seeded with 200 μL of culture medium per well in 96-well plates. The plates were incubated for 12 h to achieve cell adhesion. Three concentrations of each sampling site were evaluated: 1) Undiluted water, 2) Dilution 1:2 and 3) Dilution 1:4. In addition, two controls were used, one with injectable water and the other with culture medium (which represents 100% cell viability). The concentrations tested and controls were evaluated at 3, 6, 12, 24 and 48 h. After exposure times, 20 μL of MTT (5 mg/mL in PBS) was added to each well, and the plates were incubated for 3 h to allow the formation of formazan crystals. Later, the medium was decanted, and 100 μL of DMSO was added to dissolve the crystals. After 30 s, absorbance readings were performed at 570 and 620 nm (reference value), using a microplate reader (Thermo Scientific™ Multiskan™ FC). The amount of formazan produced is proportional to the amount of metabolically active cells and can be represented as cell viability. The experiments were performed in triplicate.

2.4. Evaluation of CYP1A1 and CYP3A4 mRNA expression by inductors

The CYP1A1 gene encodes proteins involved in the biotransformation of drugs, and cholesterol, steroids and other lipids synthesis. Its expression is mediated by the activation of AhR, as well as substances such as polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), contained in all sampling sites of the MD.

Also, CYP3A4, is responsible for drug biotransformations and endogenous compound such as ariquidonic acid, eicosanoids and steroids. For this reason the expression of this gene was determined in order to evaluate the activation of PXR by the presence of drugs.

To verify the presence of *CYP1A1* and *CYP3A4* gene, inductors were used for each of the aforementioned receptors. 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD; Accu Standard New Haven, CT, USA) was used for AhR and rifampin (Sigma-Aldrich, St Louis MO, USA) for PXR. Using DMSO (Sigma-Aldrich, St Louis MO, USA) as a vehicle.

Because the results obtained in the cell viability test showed that at sites 1, 3, 4 and 5, cell death of up to 97% occurred, this test was only performed with water from site 2, with the purpose of evaluate the activation of AhR.

The evaluation of CYP1A1 and CYP3A4 mRNA expression was performed using real time quantitative polymerase chain reaction analysis (RT-qPCR). In a six-well plate, 1×10^6 cells were seeded per well, with 2 mL of culture medium. They were incubated for 12 h for adhesion. Subsequently, the culture medium of each well was removed and water corresponding to site 2 was added, in a 1:2 dilution (where the viability was 100%). The control used consisted of a 1:2 dilution of injectable water with culture medium. For the positive controls, 10 nM TCDD and 20 μM rifampin were used. The exposure time was 24 h. Later, total RNA was prepared from SH SY5Y cultured cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's

Table 1
Groups of micropollutants present in the five sampling sites of the Madín Dam.

Micropollutant group	Pollutant	Units	Site 1	Site 2	Site 3	Site 4	Site 5
Personal care products	5-Methyl-1-H-benzotriazole	ng/L	292.7	0	0	0	134
Pharmaceuticals	Metformin	ng/L	9557	12,047	3449	5298	2526
	Penicillin G	ng/L	306.5	294.3	257	249	279
	Naproxen	ng/L	2810	1124	1141	1360.5	9156
Pesticides	Diazinon	ng/L	8.6	7.0	15	5.9	7.2
Persistent organic pollutants	PCB149	ng/L	54.2	63.6	57.2	69.4	52.3

instructions for use. The RNA obtained was quantified in a spectrophotometer at an optical density of 260 nm and the purity was evaluated by measuring the ratio of 260/280 O. D. RNA integrity was assessed by electrophoresis using a 1% agarose gel. Complementary DNA was prepared from 2 mg of total RNA using random primers and the SuperScript FirstStrand Synthesis enzyme (Invitrogen, Carlsbad, CA, USA). The polymerase chain reaction was performed in a StepOne real-time PCR system with TaqMan Universal PCR Master Mix (Applied Biosystems, Branchburg, NJ, USA) following the manufacturer's instructions for use. The relative expression genetic was quantified by the comparative threshold cycle method (CT). The probes used for *CYP1A1* and *18S* (endogenous gene) were obtained from Applied Biosystems (Branchburg, NJ, USA), with the identification numbers: Hs02382618_s1 and Hs99999901_s1, respectively. Three independent experiments are performed for statistical analysis.

2.5. Statistical analysis

The results are shown as the mean values with standard deviation. Cell viability data were analyzed by groups and exposure times. The sampling sites were compared with respect to the control for each concentration used. For this analysis, a one-way ANOVA is performed and the difference in means was performed using Tukey test. The results with a $p < 0.05$ were considered significant. Gene expression results were analyzed by Student's *t*-test, comparing the ST of site 2 with respect to the control. Analysis were carried out with the statistical program SigmaPlot version 11.0 and those results with a $p < 0.05$ were considered significant.

3. Results

3.1. Cellular viability

The results of cell viability in undiluted water of MD are shown in Fig. 2. As can be seen, in sampling sites 1, 3, 4 and 5, cell death of up to 97% occurred with respect to control group ($p > 0.05$). However, for sampling site 2 in exposure times 3, 6 and 12 h, a cell viability of SH SY5Y ranging from 20 to 50% with respect to control was observed ($p < 0.05$).

As shown in Fig. 2, a generalized death was observed in undiluted water of MD in most sampling sites (1, 3, 4 and 5). For this reason, it was decided to make 1:2 and 1:4 dilutions to see if the results were modified.

In the 1:2 dilution of the water from the five sampling sites of the Madin Dam, no significant difference was observed with respect to the controls at any time of exposure ($p > 0.05$). Also, in Fig. 3, it can be seen that the viability was similar to the controls at all MD sampling sites and at all times.

In Fig. 4, the same behavior as in the 1:2 dilution can be observed, no significant differences were observed with respect to the control at any MD sampling site and at any exposure time ($p > 0.05$). This showed that this dilution also did not affect the cellular viability of SH SY5Y.

3.2. Relative expression of mRNA from CYP1A1 and CYP3A4

Referring to the evaluation of the expression of the *CYP1A1* gene using the TCDD as inducer at 24 h, in Fig. 5, we can observe that the

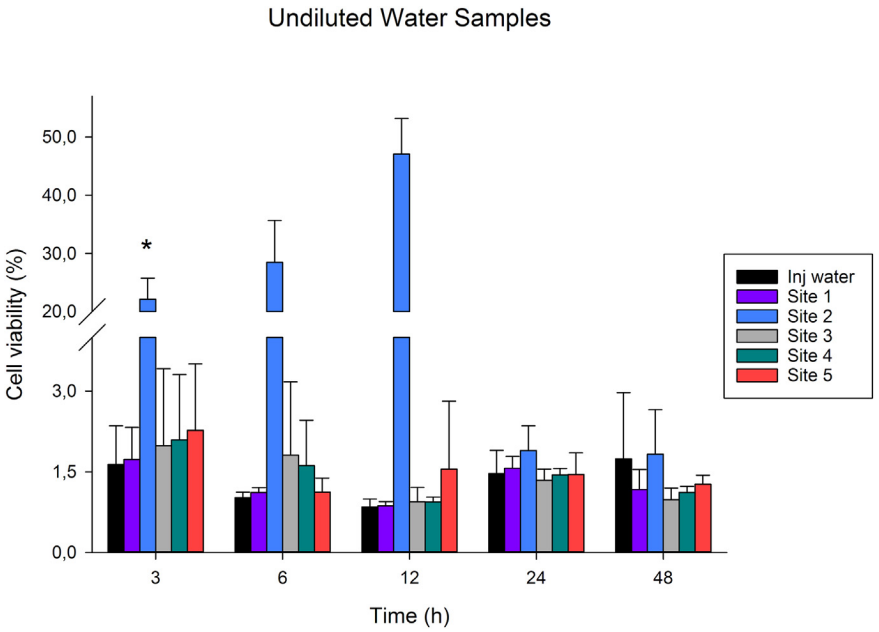

Fig. 2. Cell viability of SH SY5Y of undiluted water sampling sites from Madín Dam in different exposure times. Values are the mean of three replicates ± SE. *Significantly different from control group values, ANOVA and Tukey test ($p < 0.05$).

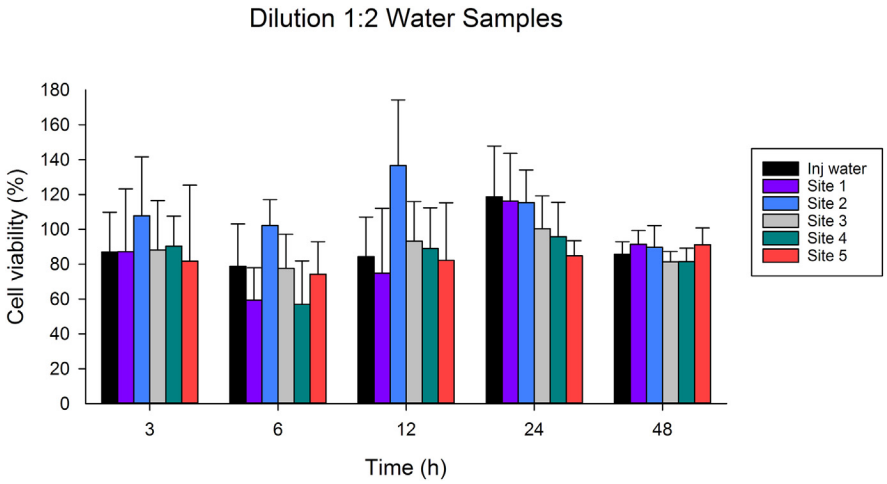

Fig. 3. Cell viability of SH SY5Y of water sampling sites from Madín Dam (Dilution 1:2) in different exposure times. Values are the mean of three replicates ± SE. After ANOVA, no significant difference was observed with respect to the control ($p > 0.05$).

expression of the gene is approximately eight times greater with the respective DMSO control ($p < 0.05$).

For the evaluation of CYP3A4, a rifampicin inducer was used at 24 h, however, no results were obtained that evidenced the expression of the gene. Being, rifampicin one of the most potent inducers of this gene, it can be concluded that the SH SY5Y cell line does not express the gene.

Once the expression of *CYP1A1* in the SH SY5Y cell line with an inducer was demonstrated, its expression was evaluated by the effect of the contaminants present in the water of the MD. Given the results reported in this study for cell viability, sites 1, 3,4 and 5 were discarded, and only the expression of *CYP1A1* for site 2 was evaluated, at a 1:2 dilution (where a viability >100%), which indicates that the cells have the necessary nutrients for optimal growth.

As observed in Fig. 6, the expression of CYP1A1 was negative approximately 7 times in the water of site 2 (1:2 dilution) with respect to the control of injectable water.

4. Discussion

The results of this study demonstrate that SH SY5Y cell line is good biomarker and predictor to evaluate the cytotoxic effects of environmental pollutants present in water bodies. The cell death obtained at sites 1, 3, 4 and 5 could be explained by the presence of some contaminants present in the Madín Dam. For example, in a study by Bermejo-Bescós et al. (2008), they demonstrated that Fe^{2+} at a concentration of 200 μM has an effect on viability and lysis of the SH SY5Y cell line. This pollutant has been identified in the Madín Dam, González-González et al. (2014), showed that at sites 1, 3, 4 and 5 concentrations of up to 1.7, 1.5, 1.4 and 5.1 mg L^{-1} were found respectively. Also, the persistent organic pollutants (POP) identified in this study in MD, have been associated with cell death of SH SY5Y. Cocco et al. (2015), found that concentrations of POP in 5 and 10 μg mL^{-1}, generated significant damage approximately between 60 and 80% of cell death, as well as depletion in the production of cellular ATP by 45% and alteration in oxidative phosphorylation and aerobic glycolysis. Other contaminants, also identified in the Madín Dam and that have been associated with the cell death of SH SY5Y are personal care products, paracetamol, chlorpyrifos, and hexachlorobenzene.

Broniowska et al. (2016), identified that personal care products such as UV filters in concentrations of 1×10^{-4} generated decreases in the viability SH SY5Y of approximately 75%, likewise the activity of lactic dehydrogenase (LDH) increased by 44%. On the other hand, chlorpyrifos in a concentration of 5–500 μM reduced up to 80% the cell viability of SH SY5Y neuroblastoma cells, in addition to increasing apoptosis and generating DNA fragmentation by >300% (Raszewski et al., 2015).

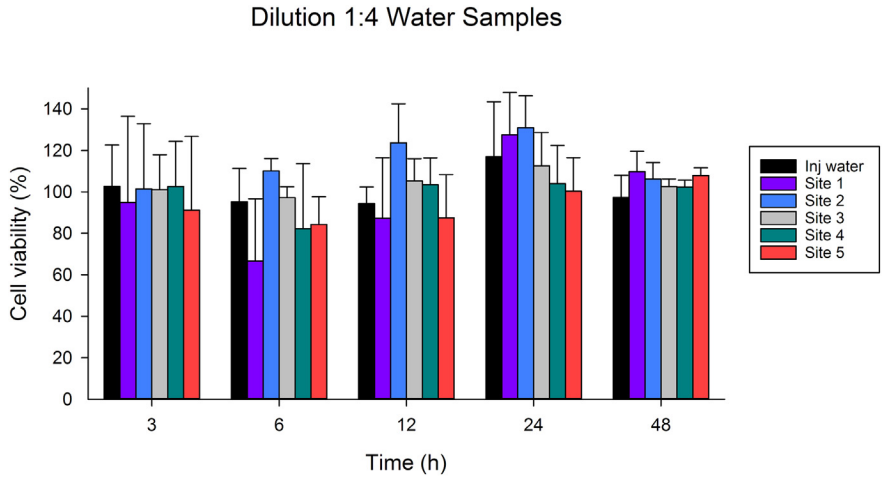

Fig. 4. Cell viability of SH SY5Y water sampling sites from Madín Dam (Dilution 1:4) in different exposure times. Values are the mean of three replicates ± SE. After ANOVA, no significant difference was observed with respect to the control ($p > 0.05$).

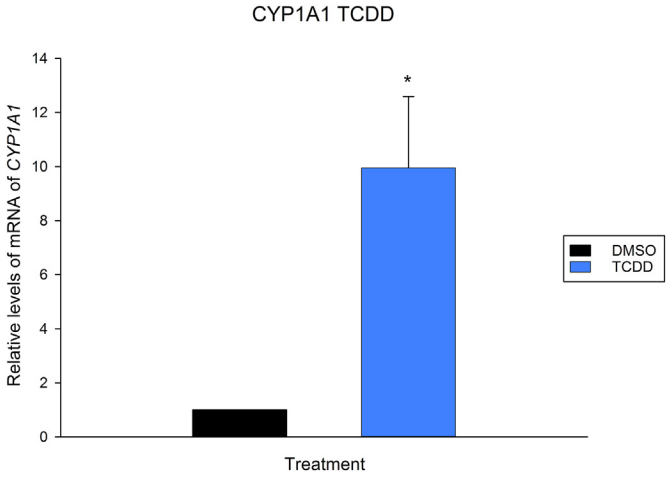

Fig. 5. Relative expression of mRNA of *CYP1A1* treated with TCDD and DMSO. Values are the mean of three replicates ± SE. Transcription levels were normalized with the *18S* expression level used as the endogenous reference gene. *Significantly different from control values ($t = 5.88$, $p = 0.004$, *4 degree of freedom*).

Chalouati et al. (2015), observed a reduction in the viability of neuroblastoma cells by up to 30% when treating cells with hexachlorobenzene in concentrations of 0.04–2000 nM. Finally, Posadas et al. (2012), they determined that acetaminophen in concentrations of 1 and 2 mM presented approximately 40% cell death and a 30% increase in LDH activity.

The cell death of SH SY5Y can also be associated with the presence of Penicillin G, a contaminant found in MD. This antibiotic at concentrations of 2000 IU, has proven to be a strong generator of neuronal hyperexcitability in mammals, and this phenomenon is involved with neurodegenrative diseases (Benítez-King et al., 2013; Leiva and Infante, 2019).

These studies are consistent with our results. At sites 1, 3, 4 and 5, presented percentages of cell death of up to 97%. However, in our study we identified that sampling site 2 presented a different behavior than the rest of the sampling sites.

SH SY5Y cells treated with undiluted water from site 2, showed an increase in cell viability of up to 50% at times 3, 6 and 12 h, suggesting that some of the compounds present in MD can stimulate survival of neuroblastoma cells. These findings could be in accordance with studies of Shan et al. (2017), who proved that pretreatment with metformin at a concentration of 0.5 mM in cells treated with 100 μM irinotecan (drug used in cancer patients), decreases the mortality of SH SY5Y. Likewise, Lu et al. (2016), demonstrated that metformin at 5 mg/ mL exerts neuroprotective effects on the degeneration of dopaminergic cells in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 20 mg kg^{-1}, a substance whose main characteristic is to produce symptoms similar to those presented in Parkinson's disease.

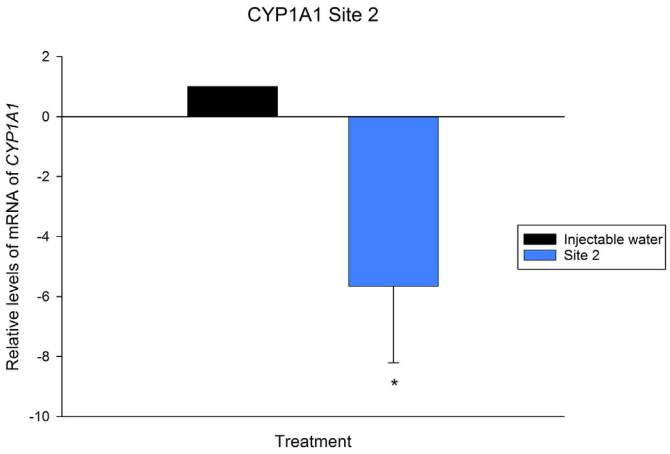

Fig. 6. Relative expression of mRNA of *CYP1A1* treated with water from site 2 of MD and control. Values are the mean of three replicates ± SE. Transcription levels were normalized with the *18S* expression level used as the endogenous reference gene. *Significantly different from control values ($t = 4.523$, $p = 0.011$, *4 degree of freedom*).

Metformin at therapeutic doses has been shown to have an antioxidant effect, in addition to anti-inflammatory and anti-apoptotic and cardioprotective properties in patients with and without diabetes who presented myocardial infarction (Higgins et al., 2019).

Cole and Frautschy (2012), demonstrated that treatment with nonsteroidal anti-inflammatory drugs such as naproxen (identified in MD), could have a protective effect on the pathogenesis of neurodegenerative diseases such as Alzheimer's, and they also slow amyloid deposition by mechanisms that remain unclear in veterans.

The results of the relative expression of the canonical genes *CYP1A1* and *CYP3A4*, were only determined in the 1:2 dilution of water from sampling site 2, because only in this dilution cell survival was observed above 100%. In the case of the CYP3A4 gene, expression in SH SY5Y was not observed. But, in the case of *CYP1A1*, the treated SY5Y SH cells showed negative gene expression.

Some of the contaminants present in MD (especially those that have a chemical structure similar to TCDD) such as PCB, have shown their ability to bind to the AhR (Calò et al., 2014; Shao et al., 2019), being able to express the *CYP1A1* gene. However, other contaminates present in MD, such as metformin, have been associated with the inhibition of AhR. Wang and Huang (2018), showed that this antidiabetic is able to block the nuclear translocation of AhR, causing inhibition in its activity. Because metformin is occurred in high amounts at site 2 (12,047 ng/ L), the predominant effect is a negative expression of *CYP1A1*.

The findings cited above, show that the contaminates present in Madín Dam, can generate effects on cell viability in a time-dependent way, since they inhibit the proliferation of SY5Y SH cells. Likewise, the negative expression of the CYP1A1 gene. In addition, this cell line can be useful to assess the risk to human health.

5. Conclusions

The contaminants present in water of sampling sites of Madín Dam generate alterations in the viability of the human neuroblastoma cells SH SY5Y. At sampling site 2, some of the compounds present in MD can protect cells from cytotoxic damage. SH SY5Y cells treated with inducer do not express the CYP3A4 gene. MD contaminants favor the negative induction of the canonical gene *CYP1A1* in SH SY5Y cells. SH SY5Y cells are good biomarkers and predictors to assess the effects of contaminates present in water bodies.

CRediT authorship contribution statement

Esmeralda Michelle Sánchez-Ocampo: Investigation, Methodology. **Guillermo Elizondo Azuela:**Investigation, Conceptualization, Methodology.**Mineko Shibayama Salas:**Investigation, Writing - original draft. **Marcela Galar-Martínez:**Writing - original draft.**Leobardo Manuel Gómez-Oliván:**Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no financial interests or personal relationships that may have influenced the work reported in this document.

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