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"EVALUACIÓN DE LA TOXICIDAD INDUCIDA POR CONTAMINANTES DE UNA PRESA DEL ESTADO DE MÉXICO EN UNA LÍNEA CELULAR DE NEUROBLASTOMA HUMANO"

T E S I S

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PRESENTA
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RESUMEN

La precipitación pluvial es la principal fuente de agua renovable que se tiene en el mundo; no obstante, la cantidad que puede ser utilizada para el uso y consumo humano es mínima y representa un factor limitante para el desarrollo mundial, así como una causa de preocupación debido a la creciente demanda poblacional y al elevado incremento de contaminantes presentes en cuerpos de agua. Diversas organizaciones han puesto especial interés en desarrollar tecnologías que permitan la remoción de estos contaminantes, pero resulta un proceso bastante complicado y costoso; por lo tanto, el enfoque actual incluye la búsqueda e implementación de estrategias para reducir la generación de dichos contaminantes e incrementar estudios que permitan establecer la relación que existe entre los contaminantes, la salud pública y el deterioro ambiental. Estudios previos han evidenciado que la exposición a contaminantes provoca alteraciones en la flora y la fauna acuáticas, mientras que en humanos se han catalogado como factores de riesgo para el desarrollo de cáncer y enfermedades neurodegenerativas. El Estado de México cuenta con una gran cantidad de cuerpos de agua, como es el caso de la Presa Madín, la cual es objeto de evaluación pues en ella se han determinado diversos contaminantes. El objetivo del presente trabajo consiste en evaluar la toxicidad del agua de la Presa Madín sobre una línea celular humana de origen neuronal (SH SY5Y), a través de la evaluación de la viabilidad celular, así como la evaluación de la expresión génica de citocromos dependientes de receptores que pueden activarse por los contaminantes presentes. De los 5 puntos de muestreo evaluados para toxicidad, únicamente uno de ellos presentó una viabilidad estadísticamente significativa en comparación con el resto, en dicho punto de muestreo se realizó la evaluación de la expresión genética de citocromos, observando una disminución estadísticamente significativa en la expresión del gen *CYP1A1*, el gen canónico de la activación del receptor para hidrocarburos arilo (AhR), el cual participa en la patogénesis de diversas enfermedades en humanos, incluidas las enfermedades neurodegenerativas.

ABSTRACT

Rainfall is the main source of renewable water in the world; however, only a minimal of this one can be used for human consumption and represents a limiting factor for world development. For this reason, there is a concern due to the growing population demand and the high increase of pollutants present in water bodies. Some organizations have put special interest in developing technologies that allow the removal of these pollutants, but this is a complicated and expensive process; therefore, the current approach includes not only searching and implementing of strategies for helping to reduce the generation of these pollutants, but also the increase of studies that allow to establish the relationship between pollutants, public health and environmental deterioration. Previous studies have shown that exposure to pollutants causes alterations in aquatic flora and fauna, while in humans they represent risk factors for the development of cancer and neurodegenerative diseases. The State of Mexico has a large number of water bodies, as the Madín Dam, which is the subject of evaluation due to its amount of some pollutants in it. The objective of this work is to evaluate the toxicity of the Madín Dam water on a human cell line of neuronal origin (SH SY5Y) through the evaluation of cell viability, as well as the evaluation of the gene expression of cytochromes dependent of receptors that can be activated by the contaminants present. Five sampling points were evaluated and the results showed that only one of them represent viability statistically significant compared to the rest. In this point an evaluation of the genetic expression of cytochromes was performed, observing a significant decrease in the expression of the *CYP1A1* gene, canonical gene responsible of the activation of the receptor for aryl hydrocarbons (AhR), and it participates in the pathogenesis of some human diseases including neurodegenerative diseases

1. INTRODUCCIÓN Y ANTECEDENTES

1.1 AGUA

Los recursos hídricos accesibles para su aprovechamiento por el hombre tienen su origen en la precipitación pluvial que, al ocurrir sobre tierra firme, se divide en dos fracciones. La primera fracción escurre superficialmente por las redes de drenaje natural como arroyos y ríos, hasta desembocar al mar o a cuerpos interiores de agua. La fracción complementaria se infiltra y circula por medio de acuíferos, que a su vez descargan a cuerpos y cursos superficiales, a través de manantiales o subterráneamente al mar.[1]

Hasta el siglo XIX el aprovechamiento creciente del agua por el hombre con la consecuente reducción gradual de los escurrimientos naturales, en general, no causó daños graves al ambiente. Sin embargo, en el transcurso del siglo XX la derivación del agua para diversos usos creció de modo acelerado, especialmente durante su segunda mitad, al grado que ahora existen porciones importantes de la superficie continental del planeta, en las cuales el ambiente ha sufrido daños graves; en casos extremos, irreparables. Varios informes publicados por la Organización Mundial de la Salud y otras agencias interesadas presentan datos alarmantes sobre la disponibilidad de agua y la asequibilidad.[1, 2]

En México, el agua subterránea es un recurso vital para el desarrollo de todos los sectores, debido a que en más del 50% de su territorio prevalecen los climas seco y semiseco. Muchas de las ciudades más importantes son abastecidas a costa del minado de los acuíferos subyacentes. Conforme éstos han resultado insuficientes, se ha incrementado gradualmente la importación de agua de áreas o de cuencas adyacentes para complementar su abasto. No obstante, esta solución es cada vez menos viable pues se presenta una gran disminución en la disponibilidad de agua y aumento en los costos asociados a su importación además de la oposición de la población rural a que sea transferida a las ciudades a costa del desarrollo local. [3]

Las actividades antropogénicas son las principales responsables de los cambios en las condiciones del suelo. Una gran cantidad de contaminantes llegan a los sistemas fluviales desde fuentes puntuales y difusas, como obras de tratamiento de aguas residuales, desbordamientos de aguas residuales, descargas domésticas, industriales y hospitalarias; así como actividades agrícolas y ganaderas, pesca comercial y actividades recreativas, principalmente.[4, 7, 8,10]

Las aguas residuales contienen grandes cantidades de compuestos orgánicos, metales, microorganismos y en menor cantidad sustancias químicas como productos farmacéuticos y de cuidado personal, microplásticos y una infinidad de compuestos no regulados. A esta diversidad de sustancias se les conoce como contaminantes emergentes; dicho término se utiliza para referirse a compuestos de distinto origen y naturaleza química, cuya presencia en el medio ambientes no se considera significativa en términos de distribución y/o concentración, por lo que pasaban inadvertidos. No obstante, ahora están siendo ampliamente detectados y tienen el potencial de provocar un impacto ecológico, así como efectos adversos sobre la salud. [5, 6, 7, 9, 11,12]

Para todas las fuentes de contaminación urbana, la descarga relativa y la composición química son inherentemente dependientes de la densidad de la población, la demografía y los tipos de actividad antropogénica dentro de la cuenca contribuyente de aguas arriba. En consecuencia, las áreas con alta densidad de población y alta cobertura de la tierra urbana contribuyen con la mayoría de los contaminantes de efluentes y xenobióticos que pueden representar una grave amenaza para la salud pública.[7, 9]

1.2 PRESA MADIN

Los cuerpos de agua se han visto afectados por la presencia de contaminantes derivados de las actividades antropogénicas principalmente, como es el caso de la presa Madín localizada en el Estado de México.[13, 14]

La presa Madín suministra agua a los municipios de Atizapán de Zaragoza y Naucalpan de Juárez en el Estado de México. Fue construida cerca del río Tlalnepantla con el propósito de controlar los flujos del río y como un reservorio de agua potable. Esta presa tiene un máximo de capacidad de 25 millones de m^3, pero por lo general almacena solo 13 millones de m^3 para poder anticipar flujos más grandes. Una pequeña parte de este volumen, aproximadamente 540–600 L/s, se bombea a la planta de purificación de agua Madín para su distribución a través del suministro de agua municipal. Adicionalmente, se utiliza para actividades recreativas como kayak, navegación y pesca de carpa común, pero en ella también se vierten descargas sanitarias de los asentamientos humanos cercanos como Viejo Madín, Nuevo Madín y Zona Esmeralda sumados a contaminantes a lo largo del propio río Tlalnepantla del cual se alimenta.[14,15]

Estudios realizados en el 2010 y 2013 mostraron que la Presa Madín contiene, entre otros contaminantes, metales pesados y medicamentos antiinflamatorios no esteroideos, en concentraciones que exceden los límites permisibles para protección de la vida. Cuando la información sobre la concentración y el tipo de contaminantes potencialmente presentes en las áreas de estudio es escasa, la calidad y salud ambiental se puede determinar con el uso de biomarcadores no específicos y/o específicos como la caracterización de algunas rutas metabólicas que pudieran estar alteradas por el efecto los contaminantes presentes en los cuerpos de agua. En particular algunos receptores para xenobióticos, como el receptor para aril hidrocarburos (AhR), receptor constitutivo de androstano (CAR) y el receptor de pregnano X (PXR), son potencialmente activados por ciertos grupos de contaminantes ambientales. Dichos receptores, al ser activados,

regulan la expresión de algunos genes de la familia de los citocromos P450.[14, 15, 16,17]

1.3 CITOCROMO P450 (CYP450)

El sistema de monooxigenasas del CYP450, el cual constituye a las enzimas de fase I, está presente prácticamente en todos los organismos eucariontes, en la mayoría de las eubacterias y en algunas arqueobacterias. En los mamíferos, el CYP450 se encuentra presente en la mitocondria y en diversos tipos de membranas celulares, siendo particularmente abundante en el retículo endoplásmico liso (microsomas). Se encuentra también presente en diferentes tejidos como el riñón, pulmón, piel, intestino, corteza adrenal, testículos, placenta, cerebro y otros. Los citocromos que comparten un 40% de homología o superior pertenecen a la misma familia. Cada familia recibe un número del 1 al 10, es decir, CYP1, CYP2, CYP3, etc.[18, 19, 20, 21, 22, 23,25]

Los xenobióticos sustratos de los CYP450s ingresan a la célula, generalmente, por difusión pasiva y en algunos casos mediante transportadores activos. Estos son identificados por los xenosensores (p.ej. AhR), los cuales promueven la expresión de los CYP450s para que realicen la biotransformación de dichos xenobióticos y su eventual eliminación, minimizando sus potenciales efectos tóxicos. [18, 19, 20, 21, 22, 23,25]

La subfamilia CYP1 contiene las isoformas 1A1, 1B1 y 1A2, que están implicados en la hidroxilación de una gran cantidad de procarcinógenos, como los hidrocarburos aromáticos policíclicos (PAH), óxidos y aminas, que son productos químicos citotóxicos, mutagénicos y cancerígenos. El gen *CYP1A1* codifica para una enzima con actividad de aril hidrocarburo hidroxilasa (AHH), siendo inducible por ligandos del AhR. En el cerebro, el AhR se expresa de forma ubicua en áreas que incluyen la corteza cerebral, el hipocampo y el cerebelo y está relacionado

con anormalidades sensoriales, motoras y cognitivas asociadas a sus ligandos ambientales. [21, 22, 23, 24, 25, 28, 30]

Diversos estudios han aportado información sobre la participación de genes que codifican para CYP450s en enfermedades neurodegenerativas, inmunológicas e incluso cáncer. Dentro de las enfermedades neurodegenerativas más frecuentes se encuentran la enfermedad de Parkinson y la enfermedad de Alzheimer. Una pequeña proporción de casos son atribuibles a mutaciones genéticas conocidas, cuyo descubrimiento está contribuyendo a conocer mejor su fisiopatología. Sin embargo, la gran mayoría se consideran debidas a la acción e interacción de diversos factores genéticos y ambientales. [24, 25]

Numerosos estudios epidemiológicos han relacionado diversos factores ambientales con mayor o menor riesgo de padecer estas enfermedades. Como claro ejemplo, se tiene la exposición a pesticidas y metales pesados, los cuales disminuyen la neurotransmisión de dopamina y aumentan el riesgo de la enfermedad de Parkinson por mecanismos asociados a disfunción mitocondrial, estrés oxidativo y deterioro en la degradación de proteínas, entre otros. El uso de modelos celulares *in vitro* ha brindado una valiosa información acerca de los mecanismos moleculares que se ven afectados por acción de algunos contaminantes ambientales. Por mencionar un ejemplo, la línea celular de neuroblastoma humano SH SY5Y la cual se usa como modelo de estudio de algunas enfermedades neurodegenerativas.[24,25,26,27]

1.4 LÍNEA CELULAR SH SY5Y

El neuroblastoma es un tumor pediátrico, a menudo mortal, del sistema nervioso simpático (SNS) derivado de células de la cresta neural embrionarias que no migran ni se diferencian adecuadamente. Durante el desarrollo embrionario, las células de la cresta neural troncal multipotentes migran ventralmente y se diferencian en neuronas para ensamblar el sistema nervioso simpático. Las

neuronas simpáticas regulan varios procesos biológicos, incluida la frecuencia cardíaca, presión arterial y la respuesta de lucha o huida. Los errores en el desarrollo de las células de la cresta neural y en las señales que regulan la diferenciación celular, pueden conducir a la patogenia del neuroblastoma.[31, 37]

2. JUSTIFICACIÓN

La legislación mundial restringe o prohíbe el uso de algunos contaminantes, sin embargo, una gran cantidad de sustancias tienen poca o nula regulación. Además, se carece de tecnología que permita la completa remoción de contaminantes en cuerpos de agua.

En los últimos años, diversos grupos de investigación han avanzado en el diseño de proyectos de investigación que ayuden a esclarecer la relación entre enfermedades poblacionales y contaminantes, pero con especial interés en enfermedades neurodegenerativas.

Por lo anterior resulta de gran importancia identificar los mecanismos que podrían explicar la asociación de contaminantes con enfermedades neurodegenerativas, proporcionando con esto las bases para invertir en programas de monitoreo, detección y evaluación, así como políticas y mejoras en la legislación que permitan reducir efectos nocivos al medio ambiente y a los organismos. [2, 13, 32, 33, 34, 35,36]

3. HIPÓTESIS

El agua de la presa Madín inducirá toxicidad y modificará la expresión del gen *CYP1A1* en la línea de neuroblastoma humano SH SY5Y, debido a la presencia de contaminantes.

4. OBJETIVOS

4.1 OBJETIVO GENERAL

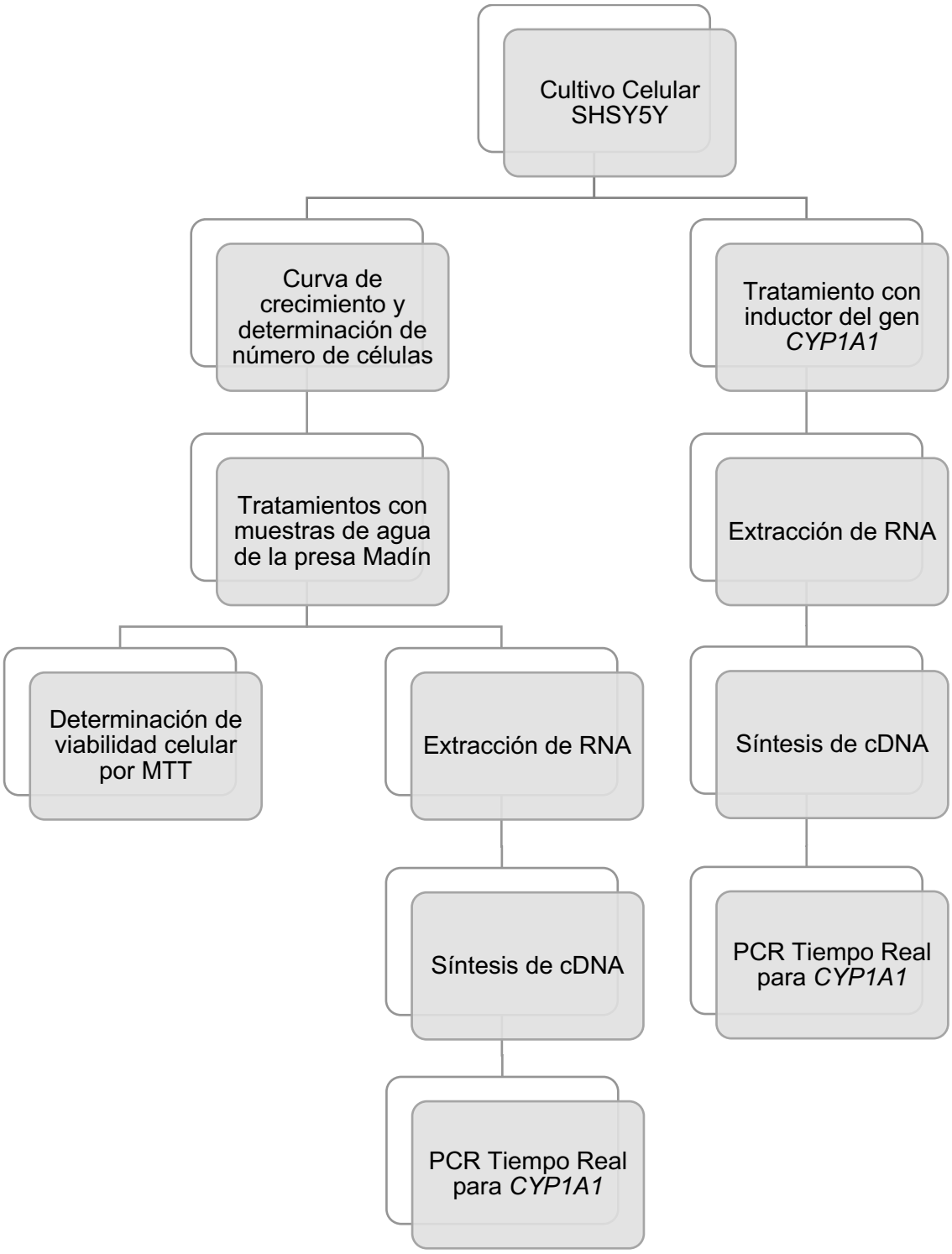
Evaluar la toxicidad y la modificación de la expresión de genes en la línea celular de neuroblastoma humano SH SY5Y inducida por los contaminantes presentes en el agua de la presa Madín.

4.2 OBJETIVOS ESPECÍFICOS

- Determinar la viabilidad de las células SH SY5Y después de la exposición a diferentes muestras de agua de la presa Madín.
- Evaluar la expresión del gen *CYP1A1* en células SH SY5Y tratadas con un inductor.
- Evaluar los niveles de expresión del gen *CYP1A1* en células SH SY5Y tratadas con muestras de agua de la presa Madín

5. METODOLOGÍA

5.1 Diseño metodológico



5.1.1 Cultivo celular

Las células SH SY5Y fueron compradas en The American Type Culture Collection (ATCC) y fueron sembradas en cajas de cultivo con 10 mL de medio Eagle Modificado de Dulbecco F-12 (DMEM F-12) con 10% de suero fetal bovino (FBS) y 1% de penicilina/estreptomicina, y se incubaron a 37 ºC con 5% de CO_2 hasta que llegaron a 70 - 80% de confluencia.

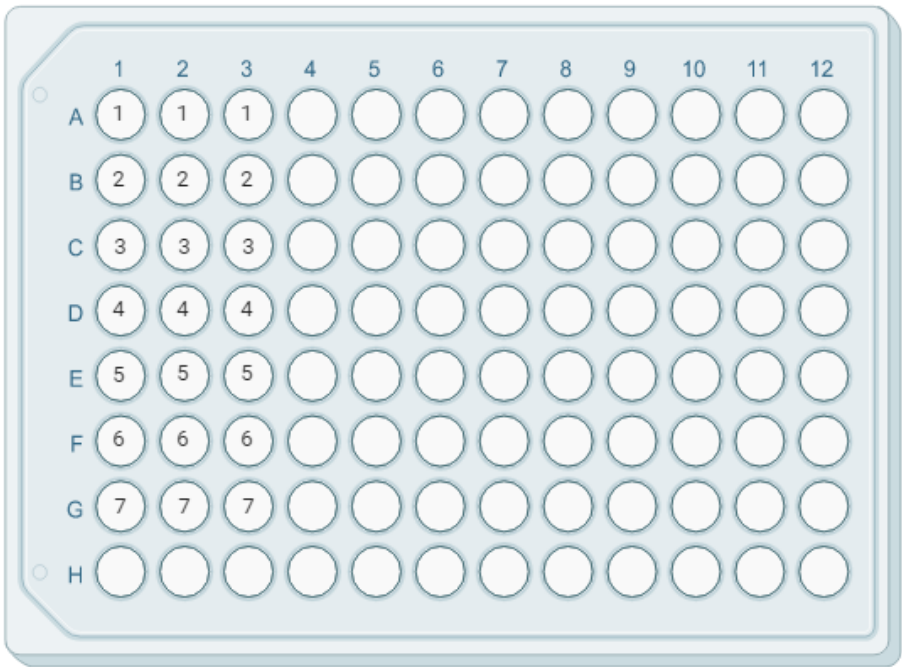
5.1.2 Curva para determinación de número de células

Una vez que se tuvieron las células en la confluencia requerida, se realizaron dos lavados con 1 mL de PBS 1x estéril a 37 ºC para eliminar la células no viables y/o residuos del medio de cultivo, se tripsinizaron usando 300 μL de tripsina/EDTA 0.05% durante 3 minutos, se adicionaron 2 mL de medio de cultivo para inactivar la actividad de la tripsina y se recolectaron las células en un tubo para centrifugarlas a 1,200 rpm por 5 minutos, se retiró el sobrenadante y el botón celular obtenido fue resuspendido en 1 mL de medio de cultivo para su conteo, usando una cámara de Neubauer. En una placa de 96 pozos se sembraron las cantidades indicadas en la tabla 1, por triplicado como se observa en la figura 1. Las células se dejaron adherir durante 12 horas y se determinó el número de células utilizando el ensayo de MTT descrito posteriormente.

Tabla 1. Determinación de número de células

Número	Cantidad de células	Volumen por pozo
1	2,500	200 microlitros
2	5,000	200 microlitros
3	10,000	200 microlitros
4	20,000	200 microlitros
5	40,000	200 microlitros
6	80,000	200 microlitros
7	160,000	200 microlitros

Figura 1. Diseño de placa para determinación de número de células

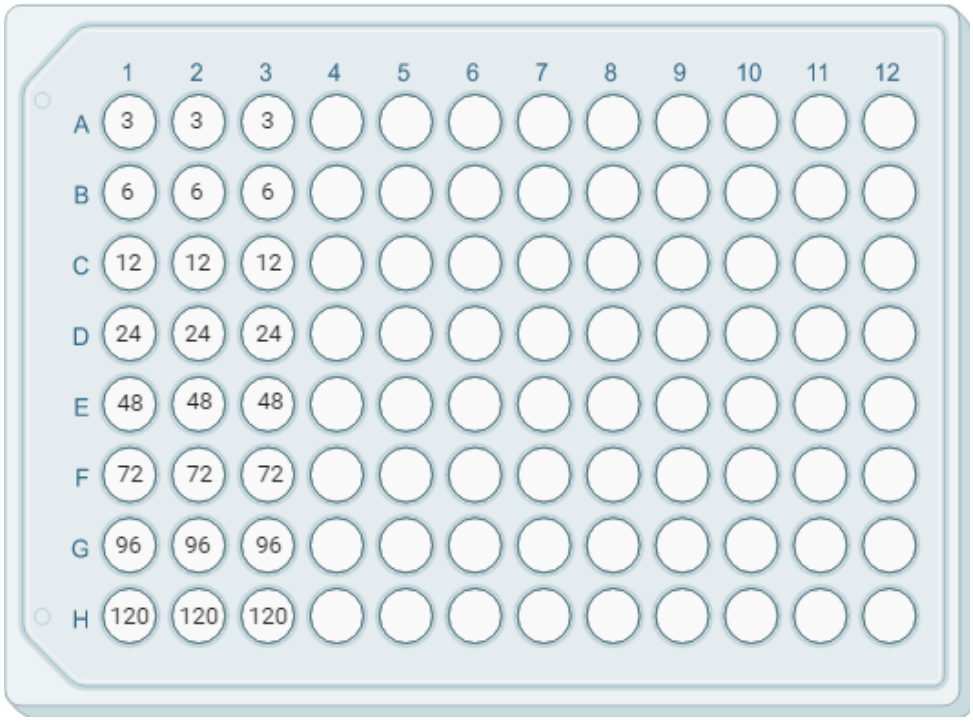


5.1.3 Curva de crecimiento celular

Cuando las células se encontraron en la confluencia requerida se sembraron para verificar el tiempo de duplicación reportado de 48 horas y observar las fases de crecimiento de la línea celular. Las células se lavaron dos veces con 1 mL de PBS 1x estéril a 37 ºC para eliminar células muertas y residuos de medio de cultivo, se tripsinizaron con 300 µl de tripsina/EDTA 0.05% durante 3 minutos y finalizado el tiempo se adicionó medio de cultivo para inactivar la actividad de la tripsina, las células fueron recolectadas en un tubo y posteriormente centrifugadas a 1,200 rpm por 5 minutos, se retiró el sobrenadante y el botón celular obtenido se resuspendió en 1 mL de medio de cultivo para su conteo en cámara de Neubauer.

Se utilizaron placas de 96 pozos sembrando 25, 000 células en un volumen de 200 microlitros por pozo, y se realizó el conteo de células viables en cámara de Neubauer a las 3, 6, 12, 24, 48, 72, 96 y 120 horas, por triplicado, como se observa en la Figura 2.

Figura 2. Diseño de placa para curva de crecimiento celular



Los resultados de las curvas indicaron que el número ideal de células para la evaluación de viabilidad celular fue de 25, 000 células por pozo.

5.1.4 Tratamientos

5.1.4.1 Viabilidad celular

Para evaluar la viabilidad celular se sembraron en placas de 96 pozos 25, 000 células por pozo, se dejaron adherir durante 12 horas con 200 microlitros de medio de cultivo y posteriormente se adicionaron los tratamientos tal como se indica en la Tabla 2.

Las diluciones realizadas se emplearon para identificar si la viabilidad se modificaba de manera estadísticamente significativa, por la reducción de nutrientes al disminuir la cantidad de medio de cultivo. Los pozos que solamente

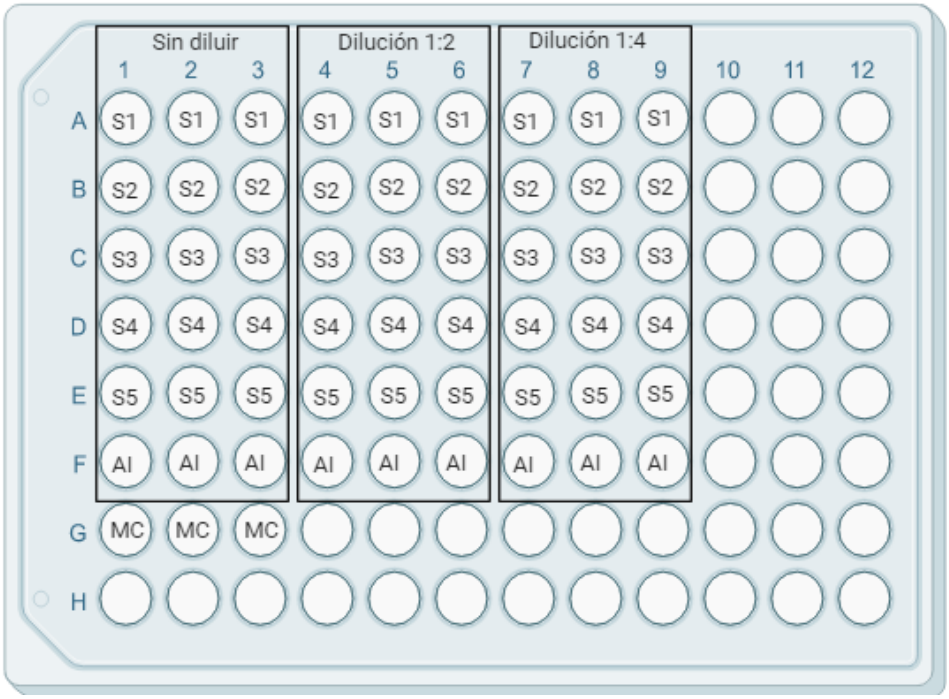
contenían células en medio de cultivo se tomaron como controles, representado el 100% de viabilidad celular.

Los tratamientos se evaluaron a los tiempos de 3, 6, 12, 24 y 48 horas, todos por triplicado tal como se muestra en la Figura 3, esto es, una placa para cada tiempo a evaluar. Terminado el tiempo de tratamiento se realizó lo descrito más adelante en el apartado Ensayo de MTT.

Tabla 2. Tratamientos para evaluar la viabilidad celular

Tratamiento	Muestra de agua sin diluir	Muestra de agua Dilución 1:2	Muestra de agua Dilución 1:4	Tiempo (h)	Volumen por pozo (microlitros)
1	Agua Sitio 1 (S1)	Agua Sitio 1 (S1)	Agua Sitio 1 (S1)	3, 6, 12, 24 y 48	200
2	Agua Sitio 2 (S2)	Agua Sitio 2 (S2)	Agua Sitio 2 (S2)	3, 6, 12, 24 y 48	200
3	Agua Sitio 3 (S3)	Agua Sitio 3 (S3)	Agua Sitio 3 (S3)	3, 6, 12, 24 y 48	200
4	Agua Sitio 4 (S4)	Agua Sitio 4 (S4)	Agua Sitio 4 (S4)	3, 6, 12, 24 y 48	200
5	Agua Sitio 5 (S4)	Agua Sitio 5 (S4)	Agua Sitio 5 (S4)	3, 6, 12, 24 y 48	200
6	Agua inyectable (AI)	Agua inyectable (AI)	Agua inyectable (AI)	3, 6, 12, 24 y 48	200
7	Medio de cultivo (MC)	-	-	3, 6, 12, 24 y 48	200

Figura 3. Diseño de placa para tratamientos de viabilidad celular



5.1.4.2 Evaluación de la expresión de citocromo con un inductor

Se sembraron un millón de células en una placa de 6 pozos y se dejaron adherir durante 12 horas con 2 mL de medio de cultivo, concluido el tiempo se retiró el medio y se adicionaron los tratamientos como lo indica la Tabla 3 en un volumen de 2 mL por pozo durante 24 horas. Se utilizó DMSO como control ya que el 2,3,7,8-Tetraclorodibenzo-p-dioxina (TCDD) se disolvió en dicho compuesto. Se realizan dos experimentos independientes siguiendo lo descrito en el apartado Expresión de mRNA de *CYP1A1*.

Tabla 3. Tratamientos para evaluar la expresión del gen con un inductor

Número de pozo	Medio de cultivo con DMSO	TCDD/DMSO (10 nanomolar)
1	2 mililitros	
2	1 mililitro	1 mililitro
3	1 mililitro	

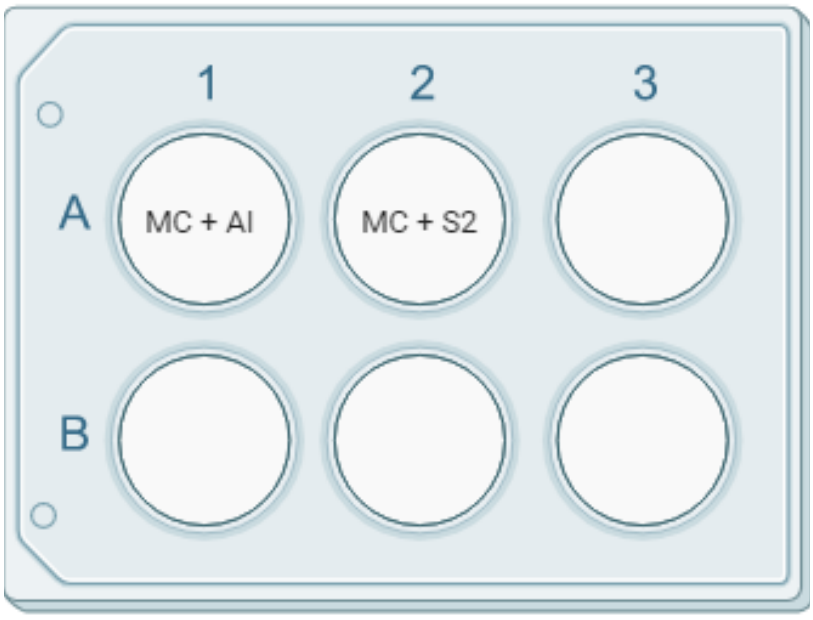
5.1.4.3 Evaluación de la expresión de citocromo con muestras de agua de la Presa Madín

En placas de 6 pozos se sembraron un millón de células por pozo en un volumen de 2 mL de medio de cultivo y se dejaron adherir durante 12 horas, transcurrido el tiempo se retiró el medio y las células fueron tratadas con agua de la Presa Madín correspondiente al sitio que presentó una diferencia estadísticamente significativa en la viabilidad respecto a los otros sitios, durante 24 horas, tal como se muestra en la Tabla 4 y Figura 4, usando como control agua inyectable. Se realizan tres experimentos independientes para el análisis estadístico.

Tabla 4. Tratamientos para cuantificar la expresión del gen con muestras de agua de la Presa Madín

Número de pozo	Medio de cultivo (MC)	Agua inyectable (AI)	Agua de la presa Sitio 2 (S2)
1	1 mililitro	1 mililitro	
2	1 mililitro		1 mililitro

Figura 4. Diseño de placa para evaluar la expresión del gen con muestras de agua de la Presa Madín



5.1.5 Ensayo MTT

Es un ensayo colorimétrico rápido, basado en la reducción de la sal de tetrazolio MTT (bromuro de 3- (4,5-dimetiltiazol-2-il) -2,5-difenil tetrazolio), sustancia de color amarillo pálido que al agregarse a las células vivas da como producto el formazán, compuesto de color azul oscuro. Esta formación se produce por la actividad de las enzimas deshidrogenasas presentes en células cuyas mitocondrias se encuentren activas. Este ensayo es empleado para determinar la sobrevivencia y proliferación de células. La cantidad de células vivas es proporcional a la cantidad de formazán producido.[39]

Una vez cumplido el tiempo de adhesión o tratamiento de las células a evaluar se retiró completamente el medio de cultivo y se adicionó medio de cultivo y 20 μl de MTT (5 mg/ml en PBS), se dejó en incubación durante 3 horas para permitir la formación de los cristales, se decantó el medio de cultivo y se agregaron 100 μl de DMSO para disolver los cristales de formazán y permitir la coloración del medio, misma que se midió en un lector de microplacas a una absorbancia de 570 nm y 620 nm, la lectura de 620 nm permite la corrección de la absorbancia dada por el efecto del colorante rojo fenol que contiene el medio de cultivo. La absorbancia utilizada para la determinación del número de células se obtiene de la diferencia de la lectura de 570 nm – 620 nm. [38]

5.1.6 Expresión de mRNA de *CYP1A1*

5.1.6.1 Extracción de RNA

Se retiraron los tratamientos y se efectuaron dos lavados con 1 mL de PBS 1x a temperatura ambiente, la placa de 6 pozos se colocó en baño de hielo y se adicionó 1 mL de Trizol a cada pozo y se incubó durante 3 minutos, el contenido de cada pozo se homogenizó y transfirió a un tubo eppendorf, los tubos se

incubaron durante 5 minutos a temperatura ambiente, se adicionaron 200 microlitros de cloroformo y se agitaron vigorosamente durante 15 segundos, se incubaron durante 1 minuto a temperatura ambiente y se centrifugaron a 13,000 rpm durante 15 minutos a 4 ºC, se transfirió la fase acuosa a un tubo eppendorf limpio y se adicionaron 500 microlitros de alcohol isopropílico, se agitaron suavemente durante 1 minuto, se incubaron 10 minutos a temperatura ambiente y se centrifugaron a 13,000 rpm durante 10 minutos a 4 ºC, se removió el sobrenadante y se agregó 1 mL de etanol al 70% y se centrifugó a 7,500 rpm durante 5 minutos a 4 ºC, se retiró el etanol, se dejó secar la pastilla y se agregaron 20 microlitros de agua DEPC libre de RNAsas y se determinó la concentración en un Nanodrop. Se evaluó también la integridad el RNA mediante una electroforesis usando un gel de agarosa al 1%, se dejó correr durante 30 minutos a 100V y se observó en el transiluminador.

5.1.6.2 Síntesis de cDNA

Se colocó en un tubo para PCR lo siguiente:

1 microlitro de random (250 nanogramos/microlitro)

2 microgramos de cada RNA obtenido

Agua DEPC para completar 13.5 microlitros

Se incubaron 5 minutos a 65 °C en un termociclador, seguidos de 2 minutos en hielo y se adicionó la siguiente mezcla de reacción a cada tubo:

4 microlitros de amortiguador 5X

1 microlitro de DTT (0.1 M)

1 microlitro de dNTP (10 mM)

0.5 microlitros de enzima Super Script II RT (200 U/mL)

Una vez adicionados todos los reactivos en los tubos de PCR se incubaron durante 40 minutos a 50°C en un termociclador.

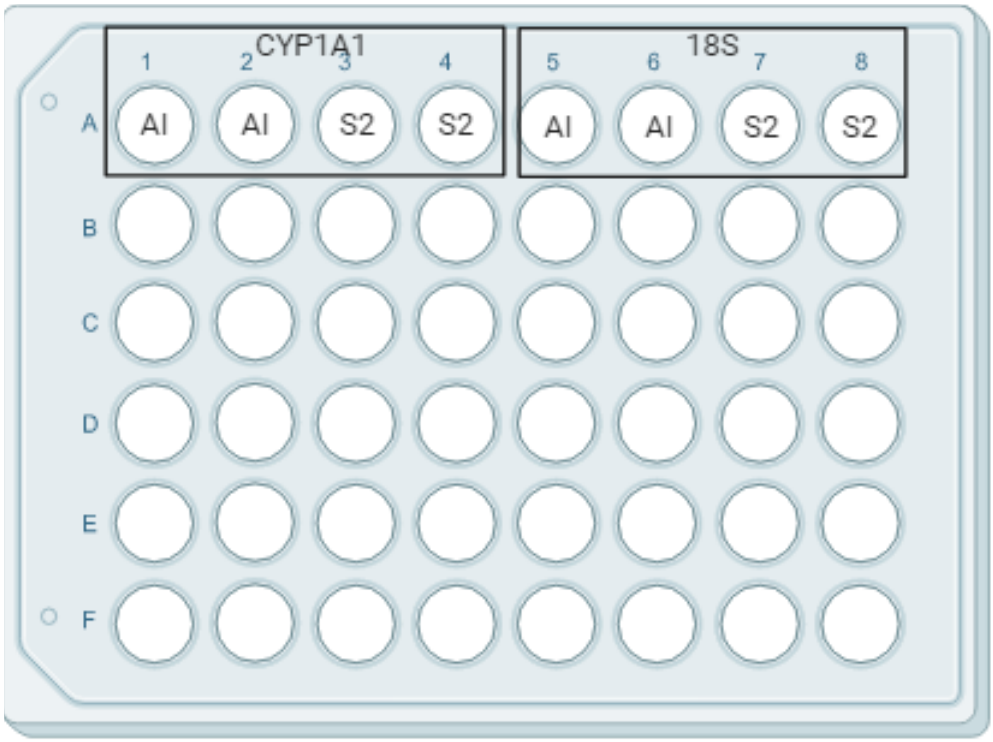
5.1.6.3 PCR Tiempo Real

Se preparó la siguiente mezcla de reacción en un tubo eppendorf

Master mix	7.5 microlitros
Agua inyectable	6.1 microlitros
Sonda Taq Man (*CYP1A1* y *18S* según sea el caso)	0.4 microlitros

Una vez teniendo las mezclas de reacción para cada gen, se colocó la tira de tubos en un soporte para tubos, y en el fondo de cada tubo se depositó 1 microlitro de cDNA por duplicado y se adicionó la mezcla de reacción, para tener un total de 15 microlitros por tubo, la distribución de las muestra se observa en la figura 5. La placa de tubos fue colocada en el termociclador para PCR Tiempo real y se determinó la expresión génica por el método ΔΔCT.[16]

Figura 5. Diseño de placa para PCR Tiempo Real



5.2 Análisis estadístico

La viabilidad celular se analizó mediante ANOVA y Tukey para evaluar las diferencias entre medias. Los resultados de expresión génica se analizaron mediante la prueba t de Student. Todas las pruebas se efectuaron con el programa estadístico SigmaPlot versión 11.0 y se consideraron significativos aquellos resultados con una $p<0.05$.

Capítulo II
Discusión de Resultados

6. ARTÍCULO DE INVESTIGACIÓN

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Manuscript Draft

Manuscript Number:

Title: Alterations in cell viability and CYP1A1 expression in SH SY5Y cell line by pollutants present in Madin Dam, Mexico

Article Type: Research Paper

Keywords: Madin Dam, Aryl hydrocarbon receptor (AhR), neurodegenerative diseases, Pollutants, SH SY5Y

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Abstract: One of the serious problems facing global development today is the availability of water, although the amount of water on the planet is quite abundant, there is only access to a minimum fraction for human use and consumption, and it is increasingly smaller due to various factors such as: the distribution of water resources, meteorological phenomena and the increase in the number of habitants. The latter has resulted in the generation of large amounts of pollutants, which has been evidenced, causing alterations in aquatic flora and fauna, and some have also been considered as risk factors for the development of cancer and neurodegenerative diseases in humans. The State of Mexico has a large number of waterbodies and the presence of pollutants has been detected in some of them, such as 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) through of the expression of the CYP1A1 gene (canonical gene). Five sampling points were evaluated and for one of them the viability was up to almost 50% with respect to the other sites, in this site the activity of the recipient was evaluated, observing a decrease in the normal expression of CYP1A1 in a statistically significant way, which opens the possibility to point out studies that establish the relationship of the contaminants present, the AhR receptor and neurodegenerative diseases.

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

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Abstract

One of the serious problems facing global development today is the availability of water, although the amount of water on the planet is quite abundant, there is only access to a minimum fraction for human use and consumption, and it is increasingly smaller due to various factors such as: the distribution of water resources, meteorological phenomena and the increase in the number of habitants. The latter has resulted in the generation of large amounts of pollutants, which has been evidenced, causing alterations in aquatic flora and fauna, and some have also been considered as risk factors for the development of cancer and neurodegenerative diseases in humans. The State of Mexico has a large number of waterbodies and the presence of pollutants has been detected in some of them, such as 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) through of the expression of the *CYP1A1* gene (canonical gene). Five sampling points were evaluated and for one of them the viability was up to almost 50% with respect to the other sites, in this site the activity of the recipient was evaluated, observing a decrease in the normal expression of *CYP1A1* in a statistically significant way, which opens the possibility to point out studies that establish the relationship of the contaminants present, the AhR receptor and neurodegenerative diseases.

Keywords: Madín Dam, Aryl hydrocarbon receptor (AhR), neurodegenerative diseases, Pollutants, SH SY5Y

1. Introduction

The water resources accessible for human use have their origin in rainfall; before man altered the hydrologic balance to meet his needs, virgin runoff sustained the entire ecosystem. Until the nineteenth century the increasing use of water by man with the consequent gradual reduction of natural runoff, in general, did not cause serious damage to the environment. However, in the course of the twentieth century the derivation of water to meet their personal needs (domestic use), for the production of food (agricultural and livestock use) and for the development of economic processes (industrial use) increased rapidly, especially during its second half, to the extent that there are now significant portions of the continental surface of the planet, in which the environment has suffered serious damage and in extreme, irreparable cases. Several reports published by the World Health Organization and other interested agencies present alarming data on water availability and affordability. It is estimated that between 17 and 19 million people in the world lack access to drinking water. (Ahkola et al., 2017; Amiri, Mazaheri, & Mohammad Vali Samani, 2019; Escalas et al., 2019; Mititelu-Ionuș, Simulescu, & Popescu, 2019; Schwan et al., 2019) (Bhati & Rai, 2017) (NOM-011-CONAGUA-2015).

In Mexico, groundwater is a vital resource for the development of all sectors, because in more than 50% of its territory dry and semi-dry climates prevail, the large reserve of water stored in regional aquifers is a resource valuable that has led to the development of arid areas but due to the growing demand for groundwater and its slow renovation, a serious ecological impact was generated in the 1960s to 1980s, derived mainly from the pollution generated by anthropogenic activities such as, discharges of industrial, hospital and domestic effluents, municipal wastewater, agricultural waste, livestock and other commercial sectors. (Dehghani, Mahmoodi, & Zarei, 2019; Gavrilescu, Demnerová, Aamand, Agathos, & Fava, 2015; Kaur, Kumar, Mehra, & Kaur, 2019; NOM-014-CONAGUA-2003, 2008; Subbiah, Karnjanapiboonwong, Maul, Wang, & Anderson, 2019; Xu et al., 2019; Yang et al., 2019).

These anthropogenic activities combined with natural sources (floods, forest fires, volcanic eruptions, among others) generated, in different water bodies around the planet the presence of contaminants of various types such as: persistent substances, heavy metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), solvents, pharmaceuticals, pesticides, personal care products and microcontaminants, to name a few; many of these are in very low concentrations, which makes it difficult to assess their presence, behavior, source and possible damage to the ecosystem. The main problem derived from this mixture of pollutants is the interactions that could occur, since substances that can be harmless in a unique way, would cause harmful effects when found in the mixture. (Chen & Liu, 2019; Crawford & Quinn, 2017; Da, Wang, Ye, & Yang,

2019; Dehghani et al., 2019; Escalas et al., 2019; Hu et al., 2019; Jiang, Ren, Hursthouse, Deng, & Wang, 2019; Kaur et al., 2019; Shao et al., 2019; Windsor, Pereira, Tyler, & Ormerod, 2019).

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 in the Mexico state; it supplies drinking water for the surrounding municipalities and is a site where various recreational activities such as fishing, sailing and kayaking are developed. The main source of contamination for this dam comes from domestic discharges from human settlements and from industrial waste and nearby businesses. (Galar-Martínez, Gómez-Oliván, Amaya-Chávez, Razo-Estrada, & García-Medina, 2010; González-González et al., 2014; Morachis-Valdez et al., 2015; Pérez-Coyotl et al., 2019, 2017).

Previous studies such as (González-González et al., 2014) show that the pollutants present in the Madín Dam induce oxidative stress in the blood, gills and muscle of *Cyprinus carpio* (common carp) cataloged as sentinel organism, (Morachis-Valdez et al., 2015) also identified changes in the physicochemical and textural properties in muscle of this same organism.

Later studies such as (Pérez-Coyotl et al., 2017) report that the pollutants present in the water of the Madín Dam also generate oxidative stress, genotoxicity and cytotoxicity in *C. carpio*, and (Pérez-Coyotl et al., 2019) it was determined that they

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, Waldron, & Gauchotte-Lindsay, 2019; Da et al., 2019; Jiang et al., 2019; Kaur et al., 2019; Mititelu-Ionuș 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. 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. 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. (A. Osawa, T. Barrocas, C. Monteiro, Oliveira, & Florêncio, 2019; Ash et al., 2017; Baltazar et al., 2014; Bondy, 2016; Crawford & Quinn, 2017; Gräns et al., 2015; Rodriguez et al., 2018; 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. 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. (Ahmed et al., 2017; Calò et al., 2014; Mejia-Garcia et al., 2013).

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, 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, 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. 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; Kaur et al., 2019; Xicoy, Wieringa, & Martens, 2017).

The aim of this study was to evaluate the toxicity of the contaminants present in the water of the Madín Dam through cell viability and the expression of the *CYP1A1* 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. (Amiri et al., 2019; Ball, Teo, Chandra, & Chapman, 2019; Blair et al., 2019; Gómez-Gutiérrez et al., 2016; Xicoy et al., 2017).

Methods

1.1 Reagents

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was obtained from Accu Standard (New Haven, CT, USA). Rifampicin and DMSO (used as a vehicle) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

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

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 Madin download

The sampling sites used for this study were previously evaluated by our research group, mainly detecting embryotoxicity, genotoxicity and cytotoxicity, this evaluated in a sentinel species. The concentrations of pollutants present in each of the Madín dam sites were obtained from the data reported by (Pérez-Coyotl et al., 2019). (Table 1).

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

Pollutants	Units	Site 1	Site 2	Site 3	Site 4	Site 5
Personal care products such as 5-Methyl-1-H-benzotriazole	ng/L	292.7	0	0	0	134
Antidiabetics such as Metformin	ng/L	9557	12047	3449	5298	2526
Beta-lactams such as Penicillin G	ng/L	306.5	294.3	257	249	279
Nonsteroidal anti-inflammatory drugs such as Naproxen	ng/L	2810	1124	1141	1360.5	9156
Pesticides such as polychlorinated biphenyls	ng/L	54.2	63.6	57.2	69.4	52.3

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.

2.3 Treatments

2.3.1 Cell viability

25,000 cells were seeded per well in a 96-well plate with 200 µL of culture medium and allowed to adhere for 12 hours, after the time 3 concentrations of the samples were evaluated by adding: 1. Undiluted water, 2. Dilution 1:2, 3. Dilution 1:4. A well with injectable water and another well with culture medium as control were also placed (it represents 100% cell viability). Dilutions were made with culture medium and the final volumes were 200 µL for each well. Treatments were performed at 5 times: 3, 6, 12, 24 and 48 hours. For each time the three concentrations were evaluated in triplicate.

2.3.2 *CYP1A1* mRNA expression

In 6-well plates, 1 x 10^6 cells were seeded per well in a volume of 2 mL of culture medium and allowed to adhere for 12 hours, after the time the medium was removed and the cells were treated with water from the Madín Dam, since the viability data were analyzed, S2 was chosen as the sampling point that could give us more information regarding the expression of the gene to be evaluated. For the treatment a 1:2 dilution was used with culture medium for 24 hours. Injectable water was used as a control, with the same dilution. The concentrations of contaminants present in S2 are reported in Table 1.

2.4 Evaluation of cell viability by MTT

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 St. Louis, MO, USA). Once the treatment time was over, the culture medium was changed and 20 µl of MTT (5 mg/mL in PBS) was added, left in incubation for 3 hours, decanted and 100 mL of DMSO was added once When the formazan crystals were dissolved, the absorbance at 570 nm and 620 nm (as a reference value) was measured in a microplate reader (Thermo Scientific™ Multiskan™ FC). The absorbance is obtained from the difference resulting from the absorbance of 570 nm - 620 nm, the amount of formazan produced is proportional to the amount of metabolically active cells and can be represented as cell viability.

2.5 Real time quantitative polymerase chain reaction analysis (RT-qPCR)

Total RNA was prepared from SH SY5Y cultured cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's 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. The integrity of the RNA was evaluated by 1% agarose gel electrophoresis. 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 relative expression genetic was quantified by the comparative threshold cycle method (CT). The probes used for *CYP1A1* and *18S* were obtained from Applied Biosystems (Branchburg, NJ, USA), with the identification numbers: Hs02382618_s1 and Hs99999901_s1, respectively. Three independent experiments were performed for statistical analysis.

2.6 Statistical analysis

The results are shown as the mean values with standard deviation. The ANOVA and Tukey test was used for cell viability to assess the differences between means. Gene expression results were analyzed by Student's t-test. All tests 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

With undiluted water samples, it was expected to obtain a low or zero viability percentage, given the effect of the contaminants present in each of the Madín Dam sites, which are reported in Table 1. For dilutions 1:2 and 1:4 for all times the percentages were close to 100%, effects of similar behavior were observed in the cells treated with injectable water, which was done to rule out that the decrease in cell viability was due to the effect of water per se, and no of the contaminants present in the dam water.

For the 3 hours of treatments it was observed that the cells treated with water from Site 2 have a viability percentage slightly above 20%, this difference being statistically significant with respect to the rest of the sites and the control of injectable water. In the case of the 1: 2 dilution a slight increase is observed in the same site 2 with a $p > 0.05$, the rest of the sites show percentages between 80 and 90% viability. For the 1:4 dilution it is observed that all values are close to 100% viability. Regarding dilutions, significant differences are were observed compared to undiluted water treatments. (Fig. 2).

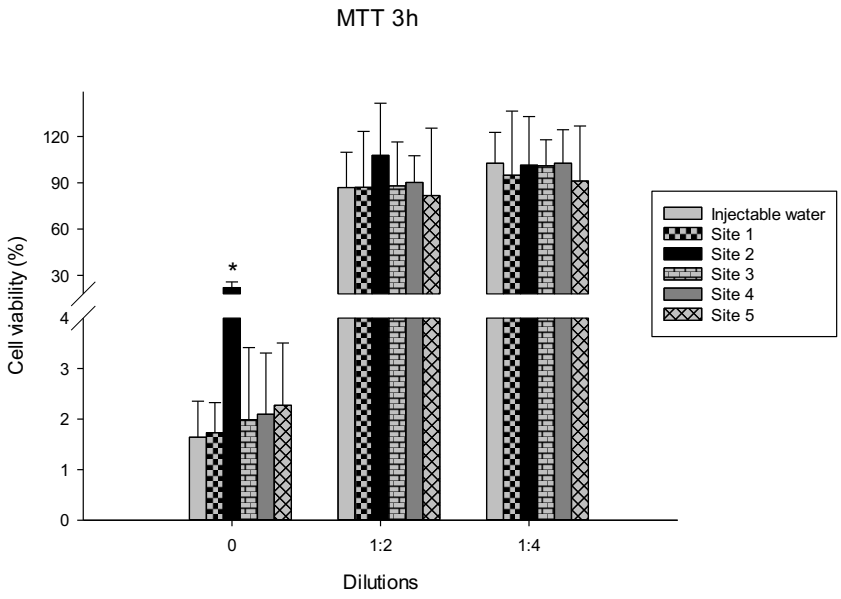

Fig. 2. Cells treated with different concentrations of water samples from the Madín Dam, first block of undiluted water samples, second 1:2 dilution block and 1:4 dilution third block, for 3 hours. Statistically significant difference $p<0.05$ for Site 2 without dilution, with respect to the rest of the sampling sites and the control of injectable water. A statistically significant difference is shown between undiluted water treatments and the dilutions tested $p<0.05$.

For the 6 hour treatments, a behavior similar to that shown at 3 hours is observed, that is, for the cells treated with undiluted water, the viability of almost 30% is shown being statistically significant with respect to the rest of the sites and wells treated with injectable water. In the 1:2 dilution there is a slight increase in Site 2 above 100%, but not for Sites 1 and 4 whose viability is close to 60%. For the 1:4 dilution, there are values close to 100% feasibility but they are slightly lower than those reported at 3 hours. Statistically significant differences are observed in the cells treated with water from the undiluted dam against the cells treated with dilutions 1:2 and 1:4. (Fig 3).

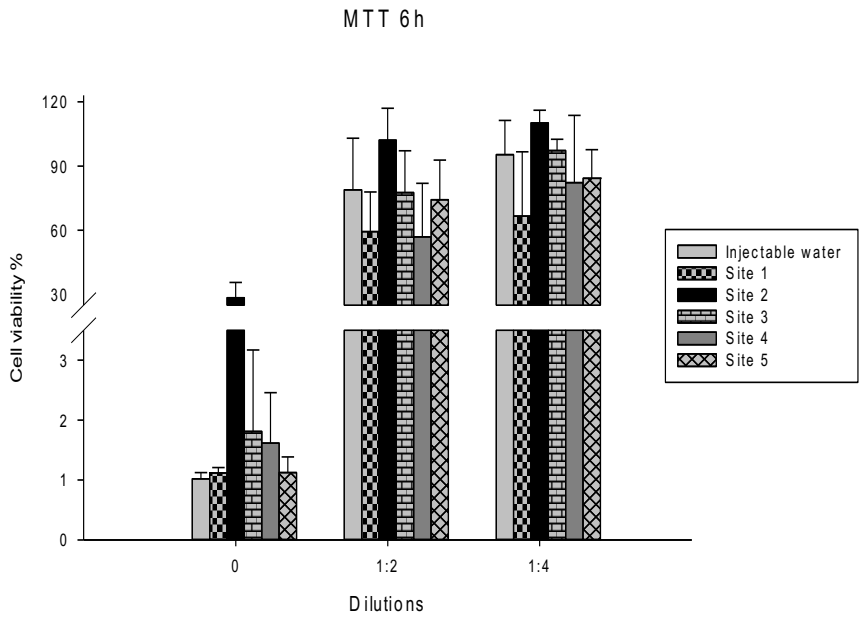

Fig. 3. Cells treated with different concentrations of water samples from the Madín Dam, first block of undiluted water samples, second 1:2 dilution block and 1:4 dilution third block, for 6 hours. Statistically significant difference $p<0.05$ for Site 2 without dilution, with respect to the rest of the sampling sites and the control of injectable water. A statistically significant difference is shown between undiluted water treatments and the dilutions tested $p<0.05$.

For the 12 hour treatments in the cells treated with undiluted water samples, a viability of almost 50% is observed for Site 2, statistically significant difference compared to the rest of the sites and the control of injectable water and a slight Increase for Site 5. For the 1: 2 dilution a slight increase in the viability for Site 2 is observed. For the 1: 4 dilution, more homogeneous and slightly higher values are shown than that shown in the 6-hour treatments. A statistically significant difference is shown between the cells treated with water from the undiluted dam and the cells treated with the dilutions tested. (Fig 4).

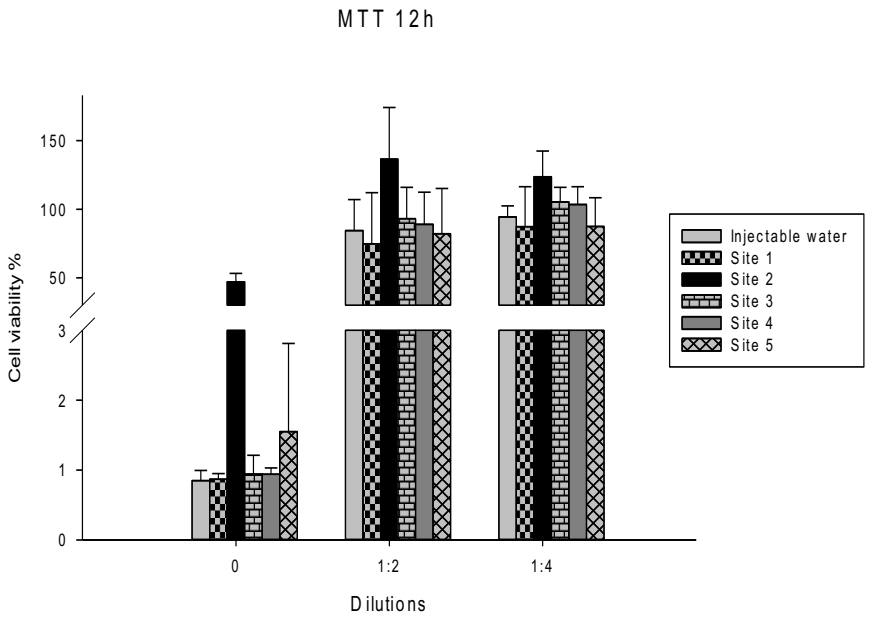

Fig. 4. Cells treated with different concentrations of water samples from the Madín Dam, first block of undiluted water samples, second 1:2 dilution block and 1:4 dilution third block, for 12 hours. Statistically significant difference $p<0.05$ for Site 2 without dilution, with respect to the rest of the sampling sites and the control of injectable water. A statistically significant difference is shown between undiluted water treatments and the dilutions tested $p<0.05$.

In the 24 hour treatments for cells treated with undiluted water samples, more homogeneous values are observed but none exceeds 2% cell viability. For the 1:2 dilution a slight increase in cell viability is observed for all sites. For the 1:4 dilution, viability values are shown higher than in the previous treatment times. Water treated cells from undiluted prey show statistically significant differences with respect to cells treated with dilutions. (Fig. 5).

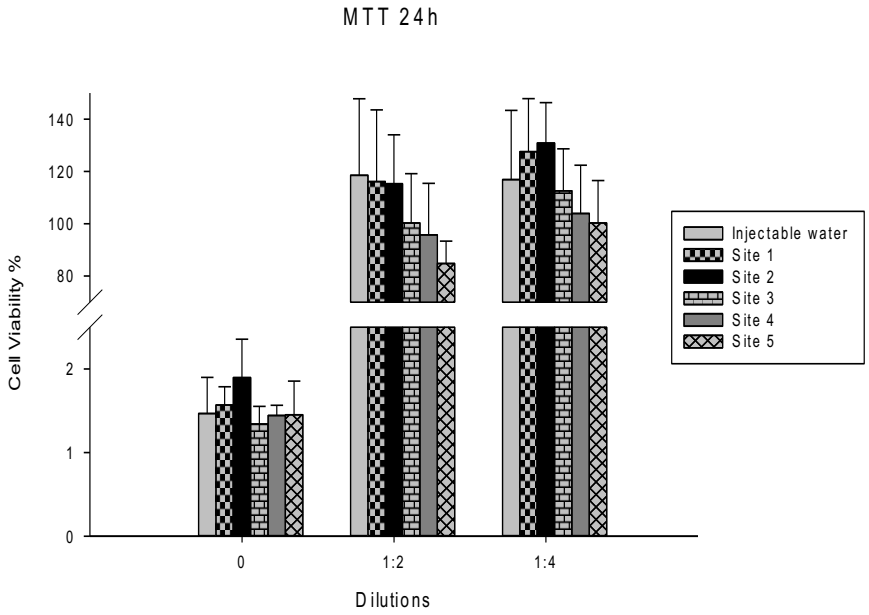

Fig. 5. Cells treated with different concentrations of water samples from the Madín Dam, first block of undiluted water samples, second 1:2 dilution block and 1:4 dilution third block, for 24 hours. There are no statistically significant differences for any of the sites between treatments. A statistically significant difference is shown between undiluted water treatments and proven dilutions $p<0.05$

For 48 hour treatments, it is observed that in the cells treated with water from the undiluted prey, a behavior similar to the cells treated for 24 hours is presented, that is, they do not exceed 2% viability. For the 1:2 dilution there are slightly lower viability percentages than in the 24-hour treatment. For 1:4 dilution the viability between sites is similar but slightly less than in the treated cells for 24 hours. A statistically significant difference is observed between the cells treated with water from the undiluted dam and the cells whose treatment was with dilutions. (Fig. 6).

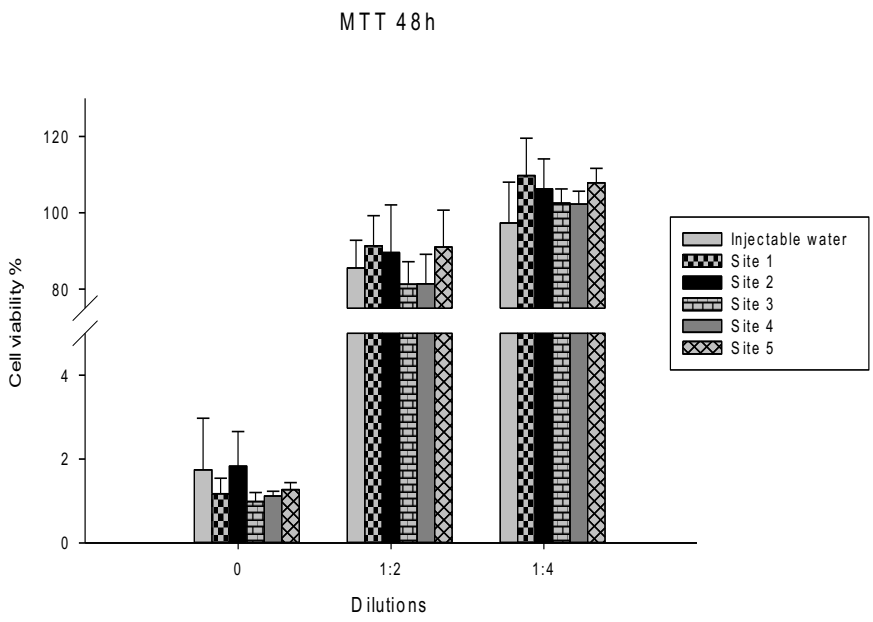

Fig. 6. Cells treated with different concentrations of water samples from the Madín Dam, first block of undiluted water samples, second 1:2 dilution block and 1:4 dilution third block, for 24 hours. There are no statistically significant differences for any of the sites between treatments. A statistically significant difference is shown between undiluted water treatments and proven dilutions $p<0.05$

Fig. 7 concentrates the viability percentages for cells treated with site 2 water and injectable water at all times (3, 6, 12, 24 and 48 hours), which allows to identify more clearly the statistically significant differences submitted for this site. Given the results it is important to put special interest in the pollutants present in this site to try to understand these changes in cell viability.

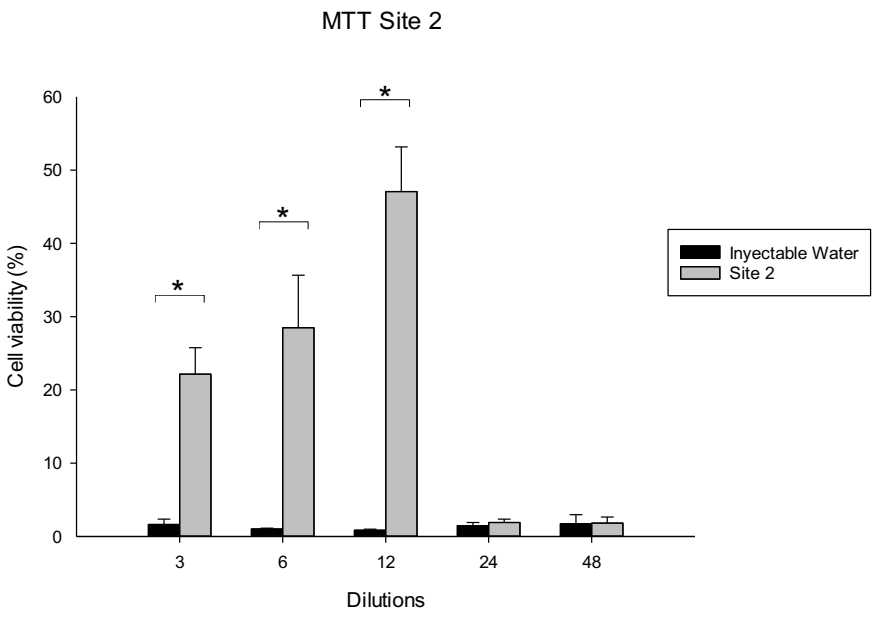

Fig. 7. Cells treated with water from site 2 at different times, statistically significant differences are observed with a $p<0.05$ between cells treated with water from site 2 and those treated with injectable water, for the times of 3, 6 and 12 hours. For the 24 and 48 hours times, there are no statistically significant differences.

3.2 Relative expression of mRNA from *CYP1A1*

Derived from the results of cell viability, the expression of *CYP1A1* mRNA was evaluated in cells treated with site 2 water using a 1:2 dilution to allow normal cell growth. The *18S* was used as an endogenous gene to normalize the expression data and as an injectable water control, giving the relative expression level of the mRNA of *CYP1A1* of 1, which was compared with the relative expression level of mRNA of *CYP1A1* for cells treated with water from the dam corresponding to S2, showing a statistically significant difference with a $p<0.01$, with an approximate

value of 6 times less than the control; Data are presented as the average of three independent experiments. (Fig. 8).

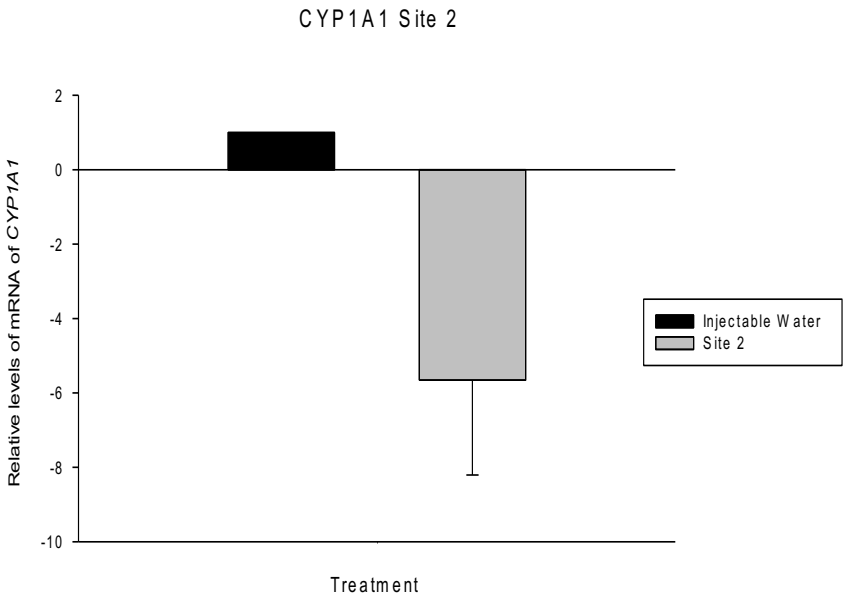

Fig. 8. Relative expression of mRNA of *CYP1A1* treated with water from site 2 of the Madín Dam and with injectable water as a control. Transcription levels were normalized with the *18S* expression level used as the endogenous reference gene. A statistically significant difference between treatments with a $p<0.01$ is shown.

4. Discussion

The effect of the viability in the cells treated with water of site 2 with respect to the rest of the sites for the case of the group of antidiabetics, could be given by the amount of metformin present, since, the concentration is markedly higher in S2 than in the rest of the sites, which could suggest that the effect of metformin in reducing reactive oxygen species allows cell viability at short times. Studies such as (Shan et al., 2017) identify that pretreatment with metformin in cells treated with

irinotecan (drug used in cancer patients) decreases its toxicity. (Lu et al., 2016) demonstrate that metformin exerts neuroprotective effects on the degeneration of dopaminergic cells in mice treated with MPTP, a substance whose main characteristic is to produce symptoms similar to those presented in Parkinson's disease. Studies have shown that metformin has antioxidant, anti-inflammatory, antiapoptotic properties and contributes to cardioprotection, among other functions. (Higgins, Palee, Chattipakorn, & Chattipakorn, 2019) In the case of non-steroidal anti-inflammatories, studies suggest that these compounds could have a protective effect on the pathogenesis of neurodegenerative diseases such as Alzheimer's, which, together with that reported for metformin, could help clarify the percentage viability presented in S2 with respect to the rest of the sites (Cole & Frautschy, 2012). For the group of personal care products, it was identified that these pollutants come exclusively from the discharges of the populations of Nuevo Madín and Viejo Madín, which is why being in relatively low concentrations and according to what is reported by (Molins-Delgado, Silvia Díaz-Cruz, & Barceló, 2015) no toxic are observed but it does represent a risk for the continuous release of these compounds.

The reduction in cell viability observed in our results for the 24 and 48 hour times, could be related to the metabolic processes generated by the pollutants, such is the case of penicillin G, a pollutant representative of the beta-lactam group, which used in studies such as (Leiva & Infante, 2019) to induce cortical excitability, this phenomenon is involved in neurodegenerative processes (Benítez-King et al., 2013). As for the PCB group, these compounds are persistent organic pollutants

with various health effects, classified as carcinogens by the International Agency for Research on Cancer; and depending on the amount of chlorine they contain, it can exert its toxic effect to a large extent by binding to the AhR receptor, since they are compounds with a dioxin-like structure or go through metabolic activation processes before exercising their toxicity in the case of PCBs other than dioxins. (Shao et al., 2019).

In the case of relative expression of *CYP1A1*, studies such as that of (Wang & Huang, 2018) have shown that metformin can act as an inhibitor of the activation of AhR in human cells, although the mechanism for this inhibition has not been clearly defined, studies suggest that it can block nuclear translocation, which is reinforced by the results obtained in the present study, well since having polychlorinated biphenyls, a greater activation of AhR was expected, although it is known that only dioxin-like polychlorinated biphenyls could act as activators of AhR, for all the above, and to expand knowledge about the mechanisms of action, studies with contaminants present at site 2 must be increased separately, since when metabolized, a considerable decrease in the quantities of activators available that maintain AhR activation. (Calò et al., 2014). As for the other groups of pollutants, no relationship has been found between the pollutants representative of each group with the AhR, but the possible interaction that could exist with other pollutants is not ruled out because they are in mixture.

With the results obtained in this work and previous studies of the effects caused by the presence of pollutants in the Madín Dam, the data that show the damage to the

ecosystem and the human being are increased, and provide the opportunity to carry out more specific studies and improve the methods of regulation and management, as well as water treatment by wastewater treatment plants, to reduce pollution of water bodies.

5. Conclusions

The contaminants present in the water of the Madín Dam have an obvious toxic effect on SH SY5Y cells, since they cause their death, but at a sampling site it was evidenced that within these pollutants substances that provide to the cells protective effects that prevent their death. It is also observed that cell viability is affected with respect to time, which would indicate that the longer the cells are exposed to contaminants, the greater the toxic effects. It is also observed that by having contaminants in the mixture, some could be negatively regulating the relative expression of *CYP1A1*, thereby reducing the biotransformation of the contaminants present and consequently their toxic effects, data that could explain the resulting changes in cell viability.

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Declaration of interests

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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7. CONCLUSIONES

De acuerdo a los resultados obtenidos podemos conlcuir que los contaminantes presentes en el agua de la Presa Madín provocaron efectos tóxicos en la células de la línea SH SH5Y pues se tiene una considerable reducción en la viabilidad celular, aunque algunos de los contaminantes pudieran tener un efecto protector que ayude a reducir el daño y muerte celular a tiempos corto; se identificó también que la exposición de 24 y 48 horas reduce significativamente la viabilidad celular hasta mas del 98%. Se determino también que la mezcla de contaminantes puede generar alteraciones en la expresión de genes como el *CYP1A1* el cual presentó una inducción negativa a las 24 horas.

8. PERSPECTIVAS

Evaluar una matriz que permita conocer cada uno de los contaminantes detectados en las muestras de agua de la Preda Madín, así como también mezclas de ellos para determinar los efectos tóxicos para los seres humanos.

Realizar estudios de toxicidad en diversas líneas celulares para ampliar la información que se tiene.

Realizar una matriz que permita evaluar el comportamiento de genes que pudieran tener relación directa con los contaminantes presentes a diferentes tiempos.

Realizar vínculos con organismos enfocados en el tratamiento y mejora de la calidad del agua.

Generar vínculos multidisciplinarios que permitan desarrollar y generar bases para el manejo de residuos y la reducción de la contaminación en cuerpos de agua.

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