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REVIEW ARTICLE

MicroRNA (miRNA) Differential Expression and Exposure to Crude-Oil-Related Compounds

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Abstract: This review summarizes studies on miRNA differential regulation related to exposure to crude oil and 20 different crude oil chemicals, such as hydrocarbons, sulphur, nitrogen, and metal-containing compounds. It may be interesting to explore the possibility of using early post-transcriptional regulators as a potential novel exposure biomarker.

Crude oil has been defined as a highly complex mixture of solids, liquids, and gases. Given the toxicological properties of the petroleum components, its extraction and elaboration processes represent high-risk activities for the environment and human health, especially when accidental spills occur. The effects on human health of short-term exposure to petroleum are well known, but chronic exposure effects may variate depending on the exposure type (*i.e.,* work, clean-up activities, or nearby residence).

As only two studies are focused on miRNA differential expression after crude-oil exposure, this review will also analyse the bibliography concerning different crude-oil or Petroleum-Related Compounds (PRC) exposure in *Animalia* L. kingdom and how it is related to differential miRNA transcript levels. Papers include *in vitro*, animal, and human studies across the world.

A list of 10 miRNAs (miR-142-5p, miR-126-3p, miR-24-3p, miR-451a, miR-16-5p, miR-28-5p, let-7b-5p, miR-320b, miR-27a-3p and miR-346) was created based on bibliography analysis and hypothesised as a possible "footprint" for crude-oil exposure. miRNA differential regulation can be considered a Big-Data related challenge, so different statistical programs and bioinformatics tools were used to have a better understanding of the biological significate of the most interesting data.

Keywords: MicroRNA, crude-oil, petroleum, environmental exposure, bioinformatics, petroleum-related compounds.

1. INTRODUCTION

Measuring the human health and environmental consequences of crude oil spills and petroleum-related pollution is a major concern in the XXI century. Between January 2019 and September 2020, different news agencies have reported at least 14 large oil spills around the world, including the Mauritius oil spill in the Indian Ocean (oil tanker, July 2020), Trans Mountain pipeline oil spill in Abbotsford, Canada (June 2020), the Norilsk diesel oil spill, Russia (industrial disaster, May 2020), and the SOTE pipeline bursts in Ecuadorian Amazon (April 2020). The accidental dispersion of petroleum-related products affects ecosystems, the health of workers and volunteers involved in clean-up operations, and nearby inhabitants [1, 2].

Crude oil is a complex mixture of paraffinic, cyclo-paraffinic (naphthenic), and aromatic hydrocarbons, also containing sulphur, nitrogen, oxygen, and metals (some are listed in Table **1**), but each crude oil sample is a unique mixture, not matching exactly in composition or properties any other sample [3]. Petroleum crude oils can be broadly classified as paraffinic, asphaltic, and mixed crude oils.

As studies on environmental exposure to crude-oil-related compounds effects are on an increase, a fast development in pollution biomonitoring, including metabolic or transcriptomic alterations after exposure, can be observed. Petroleum pollution impacts have been extensively studied in different marine, freshwater, and terrestrial fauna and flora [4], but just a few studies exist on the effects of long-term exposure on human health [5]. Biomarkers for assessing ongoing and recent massive exposure to crude oil include biliary PAHs metabolites and Ethoxyresorufin-O-Deethylase (EROD), comet assay, level of metals in blood/urine, lead isotopic ratios to trace sources, measurement of 1-hydroxypyrene in urine, and presence of PAHs DNA adducts [6]. However, in humans, it is difficult to assess petroleum-related exposure and long-term health consequences. The major vulnerable population to such exposure includes children and pregnant women [5], and those with high dependence on natural resources which may come in contact with compounds interfering in the food chain. Understanding early damage in or-

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ganisms may help us to understand better the short- and long-term effects in humans, as well as effects in fauna different than marine.

Accidental oil spills of tankers can be considered as massive but short-term exposure, as most of the time, remediation measures are taken to limit ecologic and health issues. Occupational exposure and continuous pipelines leakage near populated territories can be considered as long-term exposure, and represent a challenge for toxicological risk assessment. Exposure to petroleum-related contaminants may occur directly *via* inhalation, dermal contact [7], ingestion [8], and indirectly via freshwater resources pollution [9, 10]. However, it may be interesting to explore the possibility of using early post-transcriptional regulation as a potential novel exposure biomarker. One of the most studied post-transcriptional regulators are microRNAs (miRNAs), small non-coding RNA molecules, long average 22 nucleotides, involved in mRNA silencing, and these have been largely proposed as specific-disease biomarkers [11].

As only two studies have focused on miRNA differential expression after crude-oil exposure, this review has also analysed the bibliography concerning different crude-oil components, or Petroleum Related Compounds (PRC), exposure in *Animalia* L. kingdom, and how it is related to differential miRNA transcript levels. As miRNA differential regulation can be considered a Big-Data-related challenge, different statistical programs and bioinformatic tools have been used to analyse the most interesting miRNAs, in order to have a better understanding of the most significant data after a review of the research. However, not enough information is available to understand if miRNA-omics can be used as early petroleum exposure biomarker. This hypothesis should be confirmed in the future in *in vitro* and *in vivo* studies.

2. METHOD

Research in online libraries (PubMed) was carried out for the terms: micro RNA, crude oil, petroleum, and miRNA, obtaining only two valid results. A second research was done matching each crude oil compound mentioned in Table **1** and the words "miRNA" and "exposure" (example: [Compound_X] and [miRNA] and [exposure]). Papers regarding compounds studied in tobacco smoking were excluded.

Table 1. A summary of petroleum compounds found in bibliography and studies related to exposure and deregulated miRNAs.

Compounds	Chemical Group	Compound	References	Number of differentially expressed miRNAs mentioned	aRS
Hydrocarbon Compounds	Aliphatics	2,5-hexanedione	[1]	7	1.00
	Aromatic hydrocarbons	Benzene	[7]	48	1.01
		Benzene and Toluene	[1]	6	1.00
		Benzo(a)pyrene	[11]	215	0.80
		Benzo(a)anthracene	[1]	3	1.03
		Phenanthrene	[1]	2	1.06
		Toluene	[1]	7	1.11
	Ketones	Benzophenone-3	[3]	43	1.03
	Phenols	Hydroquinone	[1]	6	1.00
	Other Phenols	-	[1]	8	1.00
Non-hydrocarbon compounds	Metals	Aluminium	[3]	22	0.97
		Aluminium + Fluorine	[1]	22	1.00
		Antimony	[1]	10	1.00
		Arsenic	[2]	29	0.99
		Arsenic + Cadmium + Lead	[1]	12	1.01
		Arsenite + Cadmium	[1]	2	1.00
		Cadmium	[11]	250	0.76
		Chromium	[4]	54	1.01
		Cobalt	[1]	3	1.00
		Iron	[1]	3	1.00
		Lead	[11]	51	0.84
		Manganese	[1]	5	1.00
		Mercury	[3]	43	1.01
		Molybdenum	[1]	4	1.00
		Silver	[1]	2	0.00
		Titanium	[2]	21	1.00
		Uranium	[1]	4	1.00
	Sulphides	-	[2]	21	0.95

Only full-text available papers were used. Reviews were excluded in the miRNA list, but included in the "Introduction" and "Conclusions and Discussion" chapters. Non-Animalia-kingdom exposure studies were also excluded. After an accurate reading, papers were chosen according to the author's criteria.

This review proposes a new approach to evaluate the level of evidence existing regarding microRNA differential regulation after xenobiotic exposure. A lot of miRNA-target protein interaction mechanisms need to be clarified, but using a human-AI synergic approach to review how much do we know today, may facilitate new research on microRNA-omics. For this purpose, bibliographic data of each study were analysed using scite.ai (https://scite.ai/) artificial intelligence. The formula (Formula 1) to measure the impact of each reference using scite.ai, calculating a Rank Score (RS) for each paper, is provided below.

Formula 1: Rank score formula based on scite.ai data for each reference.

$$RS = \frac{S + M + C}{(S * 0.5) + (M) + (C * 1.5)}$$

In this formula, papers with a high ratio of supporting publications present a higher RS than those with contradicting publications. Articles with only mentions indicate RS= 1, no matter how many times they have been mentioned. The number of Supporting (S) and Contradicting (C) papers was taken from scite.ai, and the number of Mentions (M) was taken from the article's journal metrics when available. An analysis of papers mentioning different exposures can be found in Table **1S**, and each reference RS was included in each miRNA entry in Table **2S**. The average RS (aRS) was also calculated for each mentioned pollutant using reference's RS for each differentially regulated miRNA using IBM® SPSS® Statistics' Average function.

A hand-made list of 910 entries was created from the bibliography, including miRNAs from each table and supplemental material reported as significative differentially expressed (FC>2, p≤0.05) after exposure to single petroleum compounds (Table **1**) from *in vitro* and *in vivo* studies in Animalia L. kingdom. Each entry contains the species, miRNA name, miRNA accession, gene family, pollutant, and the tissue studied in each reference (Table **2S**). To match a single miRNA in the created list with its accession and gene family, *miRbaseConverter* R package was used [12]. Enrichment Gene Ontology - Biological Process analysis was performed on top ten most cited miRNAs in Table **2S**, using Bioconductor (https://www.bioconductor.org/) *miRNAtap* R Package [13] and *topGO* R Package [14]. After identifying and classifying the most significant GO terms for each miRNA in the top-ten list, Revigo tool (http://revigo.irb.hr/) [15] was used to create a large-similarity-semantic-based scatterplot. Raw data and the code used for this publication are available at https://github.com/CoronelVargasG/miRNAsPetroleum. IBM® SPSS® Statistics was used to analyse the frequency of miRNAs and gene families in Table **2S**. The flowchart shown in Fig. (**1**) summarizes the structure of this review.

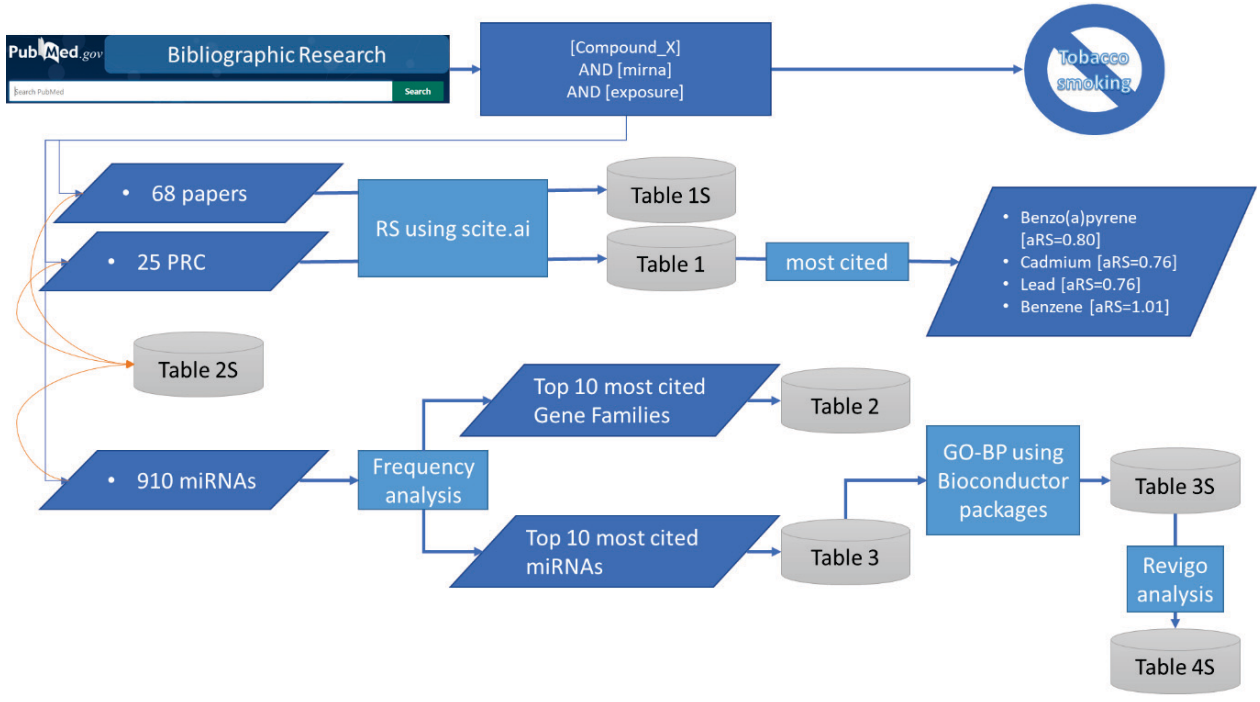

Fig. (1). Flowchart of the method used in this review. After bibliographic research and selection of 68 papers on exposure to Petroleum Related Compounds (PRC) and miRNA differential expression, different informatics tools were used to obtain information provided in different tables, including Gene Ontology - Biological Process analysis (GO-BP).

3. PETROLEUM-RELATED COMPOUNDS PRESENT IN BIBLIOGRAPHY AND STUDIES ON miRNA DIFFERENTIAL REGULATION AFTER EXPOSURE

A total of 68 articles describing exposure to 25 different compounds present in crude oil were finally chosen after the database research with the method previously explained, and can be found in Supplementary Material (Table **1S**). As it can be seen in Table **1**, most frequently mentioned compounds are Benzo(a)pyrene, Cadmium and Lead with 11 references, and the compounds with the highest number of mentioned differentially expressed miRNAs are Cadmium (250), Benzo(a)pyrene (215), Chromium (54), Lead (51), Benzene (48), and Mercury (43). Using scite.ai information, we can see that the most cited compounds have important contradictions as they present the lowest aRS: Benzo(a)pyrene=0.80, Cadmium=0.76, and Lead=0.84. The highest aRS values can be found for Toluene (1.11), Phenanthrene (1.06), Benzo(a)anthracene (1.03), Ketones (1.03) and Benzene (1.01), but low contradicting references may depend on the minor quantity of studies.

Cadmium, Benzo(a)pyrene, Chromium, Lead, Benzene, and Mercury are the most studied miRNA deregulator petroleum-related compounds.

4. MOST MENTIONED miRNAs IN BIBLIOGRAPHY: DIFFERENTIAL EXPRESSION AFTER EXPOSURE TO PETROLEUM-RELATED COMPOUNDS

The most commonly mentioned miRNA Gene Family in the bibliography is let-7, with a total of 17 references indicating exposure to 12 different pollutants, including hydrocarbon and non-hydrocarbon compounds (2,5-hexanedione, Aluminium, Antimony, Arsenic, Benzene, Benzo(a)pyrene, Cadmium, Ketones, Lead, Mercury, Phenols, and Titanium) in 4 different species (*Homo sapiens, Mus musculus, Rattus norvegicus,* and *Salmo salar*) *in vivo* as well as *in vitro*. For *in vivo* studies, the mentioned tissues are of foetal brain, bone marrow, cervix, follicular fluid, kidney, muscle tissue, placenta, plasma, skin, spinal cord, and spleen. Instead, *in vitro* studies include bone marrow and renal cells. This result indicates that let-7 Gene family is widely deregulated after PRC exposure across species and tissues.

The second most common mentioned Gene Family is miR-10, with a total of 12 different articles highlighting the differential expression after exposure to 9 different pollutants, including hydrocarbon and non-hydrocarbon compounds (2,5-hexanedione, Benzene, Toluene, Benzo(a)pyrene, Cadmium, Chromium, Ketones, Mercury and Sulfides) in 5 different species (*Branchiostoma belcheri, Drosophila melanogaster, Homo sapiens, Mus musculus,* and *Rattus norvegicus*). The other most mentioned Gene families are mir-15, mir-142, mir-24, mir-290, mir-126, mir-28, mir-30, and mir-451. More details can be found in Table **2**. The whole data related to each miRNA can be found in Table **2S**.

Top-10 Gene Families have been found differentially expressed in plasma after different exposures, except for mir-290, which may suggest that analysing the miRNA differentially regulation from these families may be a potential biomarker footprint to detect exposure to whole crude oil.

Table 2. Top-10 differentially regulated Gene Families found in reference papers after different petroleum-related compounds (PRC) exposure, in the order of the frequency of references. Different references indicate different pollutants (hydrocarbon and non-hydrocarbon), and animal species.

Gene Family	Frequency	Mentioned Members	(No) Related Pollutant	(No) Mentioned Species	References
let-7	29	let-7a-5p, let-7b, let-7b-5p, let-7c, let-7c-5p, let-7d, let-7d-3p, let-7d-5p, let-7e, let-7e-3p, let-7e-5p, let-7f, let-7g, let-7h, let-7i, let-7i-3p, let-7i-5p	(12) 2,5-Hexanedione, Aluminium, Antimony, Arsenic, Benzene, Benzo(a)pyrene, Cadmium, Ketones, Lead, Mercury, Phenols, Titanium	(4) *Homo sapiens, Mus musculus, Rattus norvegicus, Salmo salar*	[17]
mir-10	22	miR-10-3p, miR-10-5p, miR-100-5p, miR-10a-5p, miR-10b-3p, miR-10b-5p, miR-125a-3p, miR-125a-5p, miR-125b-5p, miR-99a-5p, miR-99b-5p	(9) 2,5-Hexanedione, Benzene, Toluene, Benzo(a)pyrene, Cadmium, Chromium, Ketones, Mercury, Sulfides	(5) *Branchiostoma belcheri, Drosophila melanogaster, Homo sapiens, Mus musculus, Rattus norvegicus*	[12]
mir-15	14	miR-15a-5p, miR-15b-5p, miR-16-5p, miR-195-5p,	(11) Aluminium, Antimony, Arsenic, Cadmium, Ketones, Lead, Mercury, Molybdenum, Sulfides, Titanium, Uranium	(3) *Homo sapiens, Mus musculus, Rattus norvegicus*	[8]
mir-142	13	miR-142-3p, miR-142-5p	(11) Aluminium, Antimony, Benzene, Benzo(a)pyrene, Cobalt, Iron, Lead, Manganese, Molybdenum, Titanium, Uranium	(1) *Homo sapiens*	[5]
mir-24	13	miR-24-1-5p, miR-24-2-5p, miR-24-3p	(9) Aluminium, Antimony, Benzene, Benzo(a)pyrene, Cadmium, Ketones, Lead, Manganese, Mercury, Titanium	(3) *Homo sapiens, Mus musculus, Rattus norvegicus*	[7]

(Table 2) contd....

Gene Family	Frequency	Mentioned Members	(No) Related Pollutant	(No) Mentioned Species	References
mir-290	13	miR-291a-3p, miR-291a-5p, miR-371a-5p,	(3) 2,5-hexanedione, Benzo(a)pyrene, Toluene	(3) *Homo sapiens, Mus musculus, Rattus norvegicus*	[3]
mir-126	12	miR-126-3p, miR-126a-5p,	(11) Antimony, Cadmium, Cobalt, hydroquinone, Iron, Ketones, Lead, Manganese, Molybdenum, Titanium, Uranium	(2) *Homo sapiens, Mus musculus*	[4]
mir-28	11	miR-28-3p, miR-28-5p	(9) Antimony, Benzo(a)pyrene, Cadmium, Ketones, Lead, Molybdenum, Sulfides, Titanium, Uranium	(2) *Homo sapiens, Rattus norvegicus*	[6]
mir-30	11	miR-30a-3p, miR-30a-5p, miR-30b-5p, miR-30c-5p, miR-30e-5p	(3) Cadmium, Ketones, Mercury	(3) *Homo sapiens, Mus musculus, Rattus norvegicus*	[6]
mir-451	11	miR-451-5p, miR-451a	(7) Antimony, Benzene, Cadmium, hydroquinone, Iron, Mercury, Titanium	(3) *Homo sapiens, Mus musculus, Rattus norvegicus*	[6]

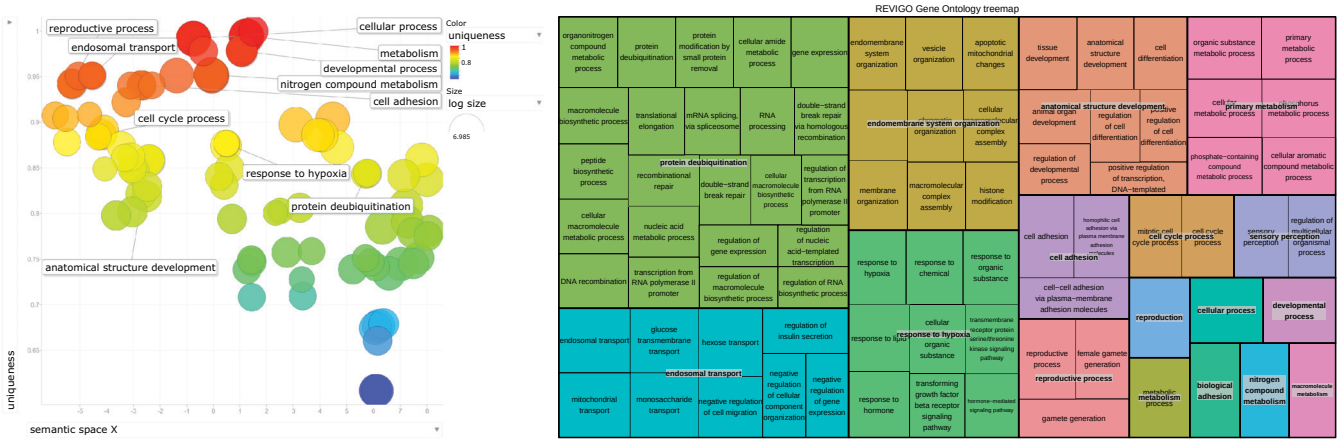

Fig. (2). Result of analysis of top-ten miRNAs GO-BP terms with Revigo tool [15]. **a**) GO terms sorted by uniqueness, with a strong cluster on cellular processes (*i.e.,* GO:0009987), and metabolism (GO:0008152) in red, and protein deubiquitination in green. **b**) GO terms treemap indicating the principal clusters of BP: protein deubiquitination, endosomal transport, endomembrane system organization, response to hypoxia, anatomical structure development, and primary metabolism.

After analysing the frequency of the most cited single miRNAs, the top-10 were found to be miR-142-5p, miR-126-3p, miR-24-3p, miR-451a, miR-16-5p, miR-28-5p, let-7b-5p, miR-320b, miR-27a-3p, and miR-346. All of these miRNAs have been found to be differentially expressed in plasma after different exposures, except for mir-346. According to Table **2**, where the most frequent Gene Family is let-7, we can see that let-7b-5p is included in this list with a frequency of 8, found in 6 different studies, referring to 6 different pollutants (2,5-hexanedione, Antimony, Cadmium, Ketone, Lead, Mercury, Titanium) in 3 different species (*Homo sapiens, Mus musculus, Rattus norvegicus*). These miRNAs may represent a possible "footprint" for crude-oil exposure as they are widely altered across different PRC and different tissues, including blood.

Enrichment analysis of Gene Ontology terms based on Biological Processes (GO-BP) was run for the ten miRNAs found in Table **3** using Bioconductor packages *miRNAtap* and *topGO.* The script can be found in the repository mentioned in the "Method" section. After identifying and classifying the most significant GO terms (Table **3S**) for each miRNA mentioned in Table **3**, Revigo tool was used to create a large-similarity-semantic-based scatterplot (Fig. **2a**) and a GO-terms treemap (Fig. **2b** and Table **4S**). According to GO Revigo-based analysis, miRNAs in Table **3** may have a strong role in 17 supercluster functions: protein deubiquitination, endosomal transport, endomembrane organization, response to hypoxia, anatomical structure development, primary metabolism, cell adhesion, reproductive process, cell cycle process, sensory perception, reproduction, developmental process, cellular process, metabolism, biological adhesion, nitrogen compound metabolism and macromolecule metabolism. A wide range of biological processes clustered by the REVIGO algorithm depend mainly on the fact that the most significant Gene Ontology estimation comes from thousands of genes predicted as targets of the Top-10 miRNAs list. Targeted genes with the major affinity for each miRNA are not listed, but their Gene ID can be easily found in the script from the repository.

Table 3. Top 10 miRNAs differentially regulated as mentioned in reference papers after exposure to Petroleum Related Compounds (PRC) in different species and tissues.

miRNA Name	Frequency	(No) PRC	(No) Mentioned Species	(No) References	Tissues
miR-142-5p	13	(11) Aluminium, Antimony, Benzene, Benzo(a)pyrene, Cobalt, Iron, Lead, Manganese, Molybdenum, Titanium, Uranium	(2) *Homo sapiens, Mus musculus*	(5) Bai W. *et al*., 2014; Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Deng Q. *et al*., 2014; Halappanavar S. *et al*., 2011	Lung, plasma
miR-126-3p	11	(9) Antimony, Cadmium, Cobalt, hydroquinone, Iron, Lead, Manganese, Molybdenum, Titanium, Uranium	(1) *Homo sapiens*	(3) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Liang B. *et al*., 2017	$CD34^+$ cells, plasma
miR-24-3p	11	(9) Aluminium, Antimony, Benzene, Benzo(a)pyrene, Ketone, Lead, Manganese, Mercury, Titanium	(1) *Homo sapiens, Mus musculus*	(6) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Deng Q. *et al*., 2014; Liu Y. *et al*., 2016; Sanders AP. *et al*., 2015; Wnuk A. *et al*., 2018	Foetal brain, Cervical swabs in pregnancy, lung, plasma
miR-451a	10	(6) Antimony, Benzene, hydroquinone, Iron, Mercury, Titanium	(2) *Homo sapiens, Mus musculus,*	(5) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Ding E. *et al*., 2016; Liang B. *et al*., 2017; Wei H. *et al*., 2015	$CD34^+$ cells, myelogenous leukemia cell line, bone marrow, plasma
miR-16-5p	9	(8) Aluminium, Antimony, Lead, Mercury, Molybdenum, Sulfides, Titanium, Uranium	(2) *Gallus gallus, Homo sapiens,*	(4) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Ding E. *et al*., 2016; Yin K. et al., 2020	Neutrophils, plasma
miR-28-5p	9	(7) Antimony, Benzo(a)pyrene, Ketone, Lead, Molybdenum, Titanium, Uranium	(2) *Homo sapiens, Rattus norvegicus,*	(4) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Deng Q. *et al*., 2014; Xu N. *et al*., 2020	Bone marrow, lung, plasma
let-7b-5p	8	(6) 2,5-hexanedione, Antimony, Cadmium, Ketone, Lead, Mercury, Titanium	(3) *Homo sapiens, Mus musculus, Rattus norvegicus.*	(6) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Lemaire J. *et al*., 2020; Piao F. *et al*., 2020; Sanders AP. *et al*., 2015; Wnuk A. *et al*., 2018	Foetal brain, Renal Proximal Tubule Epithelial cell line, Cervical swabs in pregnancy, plasma, spinal cord
miR-320b	8	(6) Aluminium, Antimony, Arsenic, Benzene, Cobalt, Lead, Titanium	(1) *Homo sapiens,*	(3) Bai W. *et al*., 2014; Cheng W. *et al*., 2019; Deng Q. *et al*., 2019	Plasma
miR-27a-3p	7	(6) Antimony, Benzo(a)pyrene, Cadmium, Lead, Manganese, Titanium	(1) *Homo sapiens,*	(4) Cheng W. *et al*., 2019; Deng Q. *et al*., 2019; Deng Q. *et al*., 2014; Lemaire J. *et al*., 2020	Renal Proximal Tubule Epithelial cell line, plasma
miR-346	6	(11) Aluminium, Antimony, Benzene, Benzo(a)pyrene, Cobalt, Iron, Lead, Manganese, Molybdenum, Titanium, Uranium	(2) *Mus musculus, Rattus norvegicus,*	(3) Brevik A. *et al*., 2012; Fay M. *et al*., 2018; Zuo J. *et al*., 2019	Whole foetus, forestomach, glandular stomach, liver, renal cortex, spleen

The principal predicted function is protein deubiquitination, which is related to its opposite process: ubiquitination (Ub). Ub is a post-translational process, highly conserved among eukaryotes, that alters proteasome and the whole cellular regulation through selective protein degradation. Ub is responsible for DNA repairing processes, DNA silencing, DNA replication, tumour suppression, transcription, stress response, receptor internalization, nuclear transport, cytoskeletal localization, and other processes that may cause serious damages to the organism if altered. The other most representative superclusters are connected to cellular stress response: endosomal transport, endomembrane system organization, response to hypoxia, and primary metabolism, which is coherent with the effects of the different PRCs that will be described further in this article.Reproductive process, developmental process, and anatomical structure development superclusters from this analysis are very interesting, as it has been reported that hydrocarbon PRCs (especially benzene) are involved in foetal damage and childhood leukemia.

The exposure to crude oil may trigger the differential expression of the mentioned miRNAs, due to PRCs synergy influencing the most significant GO-BP described in Fig. (**2**). It remains unclear if these alterations may be present after environmental pollution (*i.e.*, accidental oil spills) exposure in humans or other animals, as the only miRNA-based studies have been reported in few fish species. However, it is well known that damages caused by different components depend on the route of exposure (dermal, oral, inhalation) and the severity of environmental pollution (quantity of crude oil released). Indeed, not only biological functional alteration should be considered in future studies as a possible PRCs synergy effect, but also exposure concentration-response.

5. CRUDE OIL AND MIRNA DIFFERENTIAL EXPRESSION IN MARINE SPECIES

After the Deepwater Horizon oil spill in 2010, a major concern for marine fauna in Mexican Gulf was expressed, as

the disaster might affect spawning periods of economically important species as yellowfin tuna (*Thunnus albacares*), red drum (*Sciaenops ocellatus)*, and mahi-mahi (*Coryphaena hippurus*) [16]. In light of their critical role in early development, it was hypothesized that miRNAs might regulate dose-dependent transcriptional responses of larval *C. hippurus* in crude oil exposure. It was demonstrated that after exposure to crude oil spill and direct-well petroleum, *C. hippurus* larvae presented important morphologic differences as the larger retina, inner nuclear and ganglion diameters, as well as higher mean pericardial area compared to control. Behavioural Response was also reduced in exposed larvae. These findings were coherent with the GO-BPs of the nine significant differential expressed miRNAs (miR-499a, miR-23b, miR-734-5p, miR-301a-5p, miR-181b-5p, miR-301c-5p, miR-34b-5p, and miR-203a-3p) in the study, as most frequent BPs terms were associated with metabolic processes, tissue morphogenesis, and muscle cell differentiation including cardiovascular system development. A second study demonstrated that miR-18a, miR-27b, and miR-203a have a significant differential expression also in exposed larvae of *S. ocellatus*. After GO analysis, it was observed that miR-18a, miR-27b, and miR-203a pathways are mainly involved in embryonic morphogenesis, central nervous system development, and metabolic processes [17].

6. PETROLEUM-RELATED HYDROCARBON COMPOUNDS EXPOSURE AND MiRNA DIFFERENTIAL EXPRESSION

The major percentage of components in crude oil is represented by hydrocarbon compounds [3]. As previously mentioned in Table **1**, BaP and benzene are the compounds that refer to a major number of differentially expressed miRNAs in Table **2S**, and the most studied are 30 polycyclic aromatic hydrocarbons present in crude oil. Both components are well-known environmental carcinogens classified in IARC Group 1. The concentration of benzene in crude oil has been reported in the range between 0.01 and 1 weight percent, and BaP range between 1.2×10^{-6} to 2.8×10^{-6} weight percent [3].

Regarding miRNA differential regulation after exposure, it has been demonstrated how exposure to BaP increases miR-483-3p expression in primary cell cultures of rat hepatocytes and *in vivo* rat liver [18]. BaP can also modulate miRNA expression patterns in developing mouse embryos when paternal exposure occurs, as a significant differential expression has been reported in 102 different miRNAs [19]. In mice exposed five to ten times daily to BaP doses of 125 mg/kg, differential expression of 21 miRNAs in different organs (lung, spleen, forestomach, liver, colon, and glandular stomach) was observed, with miR-290 up-regulated in all organs except for forestomach, and miR-465 down-regulated in lung, spleen, and glandular stomach [20]. Mice treated with BaP for 28 days at 25, 50, and 75 mg/kg body weight presented not only DNA adduct formation in liver but also an upregulation of miR-34a, a downstream target of p53 gene involved in xenobiotic response [21]. BaP exposure can alter tumour-related miRNAs in breast adenocarcinoma cell lines, inducing pro-metastatic, migration, and invasiveness mechanisms [22].

The presence of BaP in saltwater ecosystems may affect fauna miRNA expression; in *B. belcheri* exposed to 0.1 mg/L, BaP have been reported in up to 58 differentially expressed miRNAs. It is of particular interest to see how these miRNAs are mainly involved in xenobiotic and cellular homeostasis, catabolic and transport processes, including pancreatic secretion, renin secretion, metabolism of xenobiotics by cytochrome P450 and other enzymes, apoptosis, T-cell receptor signalling pathway, natural killer cell-mediated cytotoxicity, and RIG-I-like receptor signalling pathway [23].

In conclusion, among the list of the 215 miRNA entries related to BaP exposure in all 11 cited studies in Table **2S** [18-29], we can find that differentially regulated Gene Families are 32 in 5 different species (*B. belcheri, H. sapiens, M. musculus, O. mykiss*, and *R. norvegicus*), with mir-290, mir-207, mir-433, mir-434, mir-10 Gene Families as the most cited. Analysing the most frequent differentially regulated miRNAs in BaP studies, we found miR-207, miR-290, miR-291b-5p, miR-292-5p, miR-346, miR-433-5p, miR-291a-3p, miR-298, miR-351, miR-376b, miR-434-3p, miR-489.

Regarding exposure to Benzene, it is an important environmental pollutant, which may cause leukemia and other lymphohematopoietic cancers [30], inhibition of erythroid differentiation in bone marrow [31], and other physiological alterations. Benzene's toxicity is related to its metabolism after exposure, as it is transformed in different compounds, including S-phenylmercapturic acid, muconaldehydes, hydroquinone (HQ), 1,4- and 1,2-benzoquinone [32].

It is plausible that miRNAs have a role in haematopoiesis regulation and haematological malignancies during benzene exposure. It has been reported that miRNA-451a and miRNA-486-5p were concisely down-regulated in *ex-vivo* $CD34^{+}$ Hematopoietic Stem Cells (HSCs) and in K562 cells (a human erythroleukemic cell line derived from pleural fluid) after treatment with Hydroquinone (HQ), a benzene metabolite, as well as in C57BL/6J mice after benzene inhalation treatment, and in bone marrow from patients with chronic occupational benzene poisoning. Moreover, miRNA-126-3p and miRNA-6089 were also reported as downregulated after HQ exposure in CD34+ HSCs [33]. Differential expression of miR-451a in mice's Lin−HSCs after benzene inhalation treatment was confirmed in another study [34], which also indicated other 22 differential expressed miRNAs, including let-7i-3p. Occupational benzene exposure has also shown a miRNA differential regulation in patients' peripheral blood, specifically miR-34a, miR-205, miR-10b, let-7d, miR-185, miR-423-5p-2, miR-133a, miR-543, miR-130a, miR-27b, miR-223, miR-142-5p, and miR-320b [35]. In another study, miR-34a has been proposed as a biomarker for benzene toxicity, without analysing other metabolites, like HQ concentration. This miRNA is also involved in cell apoptosis observed *in vitro* after 1,4-benzoquinone (another benzene metabolite) exposure in U937 (histiocytic lymphoma cell line) [36].

In humans, plasmatic microRNA expression profiles from chronic occupational exposure to benzene presented a miR-122-5p significant differential expression compared to non-exposed subjects (*i.e.*, not workers) and benzene-exposed but not poisoned workers. Moreover, miR-24-3p, miR-221-3p, and miR-638 were differentially regulated with a fold change between 1.82 and 3.93 ($p<0.01$) across benzene-poised, benzene-exposed, and not-exposed subjects [37]. Lymphocytes from peripheral blood collected from petrol station workers presented a positive correlation between miR-221 differential expression and benzene exposure. It has been suggested that miR-221 is involved in several malignancies, and it has been consistently reported to be significantly deregulated in the blood cells of patients with leukemia [38].

In conclusion, among the list of the 48 miRNA entries related to benzene exposure in all 7 cited studies in Table **1S** [32, 34-39], the most mentioned miRNA was found to be miR-451a, followed by miR-34a, and miR-486-5p, which are also included in Table **3**. The differential expression of miR-451a occurs in two different species (*h. sapiens* and *m. musculus*) after benzene exposure [33, 34].

Other hydrocarbon compounds present in crude oil and found in bibliography as miRNA deregulators are 2,5-hexanedione [40], Toluene [39, 41], Benzo(a)anthracene [42], Phenanthrene [43], and Benzophenone-3 [44, 45]. Due to the less quantity of references, it is hard to evaluate their effects when crude oil exposure occurs. However, these compounds have been included in Table **2S**, as explained in Tables **1**-**3**.

7. EXPOSURE TO PETROLEUM-RELATED NON-HYDROCARBON COMPOUNDS

Sulphur compounds, including thiols, sulphides, disulphides, and thiophenes, are the most representative non-hydrocarbon compounds present in crude oil (from >0.1% to 10% by weight). Only a few studies related to sulphur compounds exposure and miRNA differential regulation have been found.

Hydrogen Sulphide (H_2S) is the only non-hydrocarbon and non-metallic compound which has been studied for its massive exposure effect on miRNA differential expression. H_2S can be beneficial in low doses and toxic in high doses; it can act as a physiological endogenous signaling molecule and also as a highly reactive and toxic xenobiotic gas, especially for the cardiovascular and the nervous system [46]. Environmental H_2S sources include releases from volcanic eruptions and geological formations associated with natural gas and other fossil fuels; this gas is a well-known hazard to petroleum and natural gas extraction personnel [47], but until today, there have not been reported high levels of H_2S during crude-oil accidental spills. Background H_2S air concentrations typically range between 0.11 ppb and 0.33 ppb, although concentrations in urban areas can be as high as 1 ppb. Prolonged exposure to concentrations higher than 2-5 ppm may cause nausea, headaches, or loss of sleep in humans, and concentrations higher than 500 ppm cause collapse and possibly death in 30 minutes [48]. It has been demonstrated that H_2S upregulates mir-16-5p to reduce the expressions of RAF1 and PiK3R1-AKT in the neutrophil *ex vivo G. gallus* model, decreasing respiratory burst levels [49]. The effects of H_2S and other sulphuric compounds in miRNAs differential regulation may be limited to the exposure type (occupational *vs.* non-occupational). The most common atmospheric pollutants reported after oil spills are hydrocarbons, particulate matter, ozone, carbon monoxide, and nitrogen oxides [50].

Metallic compounds are present in petroleum only in traces: Calcium (500-50,000 ppm), Aluminium (200-20,000 ppm), Magnesium (200-10,000 ppm) and Titanium (100-5,000 ppm) have the highest concentrations among metals, while Cadmium (0.003-0.027 ppm), Chromium (0.0023-0.64 ppm), Lead (0.17-0.31 ppm), and Mercury (0.02-30 ppm) are present at lower concentrations [3]. As indicated in Table **1**, the most frequent studies related to miRNA differential regulation are focused on Cadmium [51-61] (aRS = 0.76, 250 miRNA entries and 11 references), Chromium [62-65] (aRS = 1.01, 54 miRNA entries, and 4 references), Lead [55, 58, 66-74] (aRS = 0.84, 51 miRNA entries, and 11 references), and Mercury [58, 71, 75] (aRS = 1.01, 49 miRNA entries, and 3 references). Metallic compounds are trace elements in crude oil, but they are important constituents that affect the refining and production operations, and metals may also be found under detection limits in oil spills. No effects of Cadmium, Chromium and Lead (the most cited metals in Table **2**) on human health after oil spills have been reported. However, it has been hypothesized that Mercury may enter the atmosphere through petroleum-derived products combustion [76, 77]. High levels of Manganese may be found in drinking water, and high concentrations of Vanadium, Barium, Cobalt, and Molybdenum in topsoil may be present near petroleum extraction fields [9].

Other metals present in crude-oil and found in bibliography as miRNA deregulator are Aluminium [55, 78-81], Antimony [54], Arsenic [53, 62, 68, 82, 83] Arsenite [84], Cobalt [53], Iron [55], Molybdenum [55] Silver [69], Titanium [53, 85], and Uranium [53], but due to the less quantity of references, it is hard to evaluate their effects on crude-oil exposure. However, these compounds were included in Table **2S** as explained in Tables **1**-**3**.

More studies need to be carried out regarding the effects of metallic compounds present in crude oil, and we can only hypothesize the synergic effects of hydrocarbon, non-hydrocarbon and metallic compounds based on studies mentioned in Table **2S** and the analysis in Fig. (**2**). The role of miRNA differential expression of trace metals in crude oil remains unclear.

8. DISCUSSION AND CONCLUSION

Information regarding crude-oil exposure and miRNAs differential regulation is extremely limited. However, it is interesting to explore the possibility of using a dataset of high-

Table 4. Sequence variation among different species of miRNAs. The variation is written as nucleotide in lowercase.

miRNA	Species	Sequence	No. of Sequence Variation
miR-142-5p	hsa-miR-142-5p	CAUAAAGUAGAAAGCACUACU	0
	mmu-miR-142-5p	CAUAAAGUAGAAAGCACUACU	
miR-451a	hsa-miR-451a	AAACCGUUACCAUUACUGAGUU	0
	mmu-miR-451a	AAACCGUUACCAUUACUGAGUU	
miR-16-5p	gga-miR-16-5p	UAGCAGCACGUAAAUAUUGGuG	1
	hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGcG	
miR-28-5p	hsa-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	0
	rno-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	
let-7b-5p	hsa-let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU	0
	mmu-let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU	
	rno-let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU	
miR-346	mmu-miR-346	UGUCUGCCcGAGUGCCUGCCUCU	1
	rno-miR-346	UGUCUGCCuGAGUGCCUGCCUCU	

ly cited differential regulated miRNAs after PRCs exposure to predict what may be the most dysregulated miRNAs after crude oil exposure across different species [86]. *In vitro* and *in vivo* experiments are needed to confirm the biomarker potential of the mentioned gene families and individual miRNAs. Some limitations for the proposed approach should be considered as this review and the *in silico* analyses heavily depend on the number of studies and citations present in the bibliography. The result may change if more information is available in the future.

The top-ten differentially regulated miRNAs here proposed were the most observed in different species in different studies. There is evidence of miRNAs cross-species variation in terms of sequence and expression within animal species and tissues, although variation in miRNAs' targets is greatly conserved across mammals [85]. In Table **3**, all mentioned species are mammals, except for *G. gallus*. This may limit the reliability of the result for non-mammal species, as it occurs for the marine species mentioned by Xu *et al*. (2018).

Among the top-10 miRNAs mentioned in Table **3**, sequence variation is appreciated for different species, only for miR-346 and miR-16-5p which have 1 variation each, as shown in Table **4**. With the available information, it is not possible to understand if highly conserved miRNAs may be more reliable as exposure biomarkers.

Different PRC influence differently the expressions of miRNAs mentioned in Table **3**. Considering that hydrocarbons represent the major percentage in crude oil, and that they are the principal chemicals studied after environmental accidental pollution, we can hypothesize that benzene, BaP and other hydrocarbons may have a decisive role in miRNA deregulation and biological processes alteration after crude oil exposure.

Metallic PRCs exposure also plays an important role in miRNA differential expression when studied as single compounds, but it remains unclear if they have a real influence on fauna and human health. Metallic PRCs are present at low concentrations in crude oil, so environmental exposure may be limited. Potentially, the most significant deregulation may be linked to Cadmium, Chromium, Lead, and Mercury, or to their synergy effects, especially due to their ability to enter the food chain and water supplies.

The list of top-10 miRNAs (miR-142-5p, miR-126-3p, miR-24-3p, miR-451a, miR-16-5p, miR-28-5p, let-7b-5p, miR-320b, miR-27a-3p and miR-346) created according to recent knowledge may be hypothesised as a possible "footprint" for crude-oil exposure in different species as they are widely altered across different PRCs, and in different tissues including blood. Petroleum is a complex mixture that includes a high quantity of toxic and cancerogenic compounds for humans and other animals. Early biomarkers should be developed to shelter public health, especially in populations exposed to crude oil spills. In the bibliography, only two studies have been found to be focused on miRNA differential regulation in animals after crude oil exposure; not enough information is available to understand if miRNA-omics can be used as a biomarker for this purpose.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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