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Predicting Chemical Toxicity in Rivers Near Electricity Station Outlet Discharges Using Quantitative Structure-Activity Relationship (QSAR)

Hasan Khadim Nimr✉ | Maitham A. Sultan | Njah K. Nimr

Environment and Water Directorate, Ministry of Science and Technology, Baghdad, Iraq.

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ABSTRACT

In this study, chemical toxicity prediction was conducted using in silico approaches due to their importance for human health and environmental concerns. Analysis of Tigris River samples near a power station outlet revealed ten compounds, with three identified as toxic by in silico tools. TOXTREE software classified three compounds as high hazards, including heavy aromatic naphtha, light aromatic naphtha, and naphthalene, which was corroborated by QSAR database analysis. QSAR data indicated positive Ames tests for eight naphtha derivatives, suggesting their mutagenic potential. Molecular docking demonstrated strong binding affinity (-6.6 kcal/mol) between naphtha and cytochrome p450, crucial for xenobiotic metabolism, indicating potential interference with detoxification processes. This study highlights the utility of in silico methods in identifying and assessing environmental chemical hazards, emphasizing the importance of monitoring and mitigating toxic pollutants. Further investigation into the long-term environmental impact and bioaccumulation potential of these identified toxic compounds is warranted to ensure comprehensive risk assessment and management.

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INTRODUCTION

Numerous environmental compartments include an enormous amount of chemical substances and their metabolites, which damage the environment and pose a risk to living systems. Ecologists are strongly concerned about pharmaceuticals and their metabolite wastes discovered in rivers, sewage effluents, streams, and surface, ground, and drinkable water due to the increase in the consumption of human and veterinary medicines (Roy & Kar, 2016). The probability that humans and wildlife will be exposed to potentially hazardous compounds is rising in direct proportion to the number and volume of manufactured chemicals that are finding their way into the environment (Mansouri et al., 2018). Pharmaceuticals primarily enter the environment through hospital, domestic, and industrial wastes (Roy & Kar, 2016). REACH, which was implemented in 2007, is a versatile chemical assessment test that the European Union has developed to examine chemicals worldwide (Rim, 2020). Bioavailability, transport, absorption, permeability, and persistence of chemicals in the body and environment are the most commonly employed chemical attributes in toxicological and exposure studies and risk assessment (Mansouri et al., 2018). Chemical testing has recently seen an advancement in in silico technologies, which rely on computer modeling or simulation. The terms “in vivo” and “in vitro,” which are more widely used in biology, are related to the new term “in silico,” which is typically used to describe research performed with computers. Computational bioinformatics,

*Corresponding Author Email: *hassaneetaby@gmail.com*

often known as in silico pharmacology, is a fast-expanding discipline. It demonstrates how to use this information to develop computer simulations or models that can be employed to predict outcomes, build hypotheses, and ultimately lead to therapeutic discoveries (Mansouri *et al*., 2018). Animal testing has been the mainstay of the process for determining the hazardous effects of compounds. At least thousands of animals must be sacrificed in order to obtain all of the toxicological results for one medication relying on laboratory animals. One of the most significant barriers in evaluating drugs that are known to be commercially available both domestically and internationally is the enormous expense and duration of animal testing (Rim, 2020). The water of the Tigris River is contaminated due to the spread of toxic chemicals from human activities, including residence, hospital, and industrial factory wastewater, that are discharged into the river untreated, passing through Baghdad city and endangering the ecosystem that supports plants and other living beings (Oleiwi & Al-Dabbas, 2022). The cytochrome P450 (CYP) enzymes establish a vast superfamily of heme proteins able to metabolize a large number of exterior and interior molecules. A number of 10 CYP models are in charge of the metabolism of xenobiotics in creatures. Pesticides, herbicides, heterocyclic and aromatic amines, polycyclic aromatic hydrocarbons, and almost all medications are metabolized by the CYPs. Ligands for CYP enzymes can function as inhibitors to stop the turnover of substrates or as substrates, like those that the enzyme has metabolized (Raunio, 2011b). This study aims to predict the toxicity of some chemicals found in the samples collected from the Tigris River using bioinformatic tools and their genetic influences. It also uses molecular docking to find the ability of cytochromp450 to react with toxic aromatic materials by detecting binding energy and affinity between the enzyme and aromatic cyclic materials.

MATERIAL AND METHOD.

Sampling

Twenty water samples were collected from the discharge outlets of the South Baghdad gas power station. The reason for sampling at this site is that the wastewater from this station, resulting mainly from the washing of tanks, could be regarded as a vast potential source of environmental contamination, particularly by hydrocarbons and related compounds. This suite of chemicals, including aromatic naphthas and naphthalene, was chosen based on their known occurrence in industrial discharges and for having some established toxicological profiles that include mutagenic and carcinogenic potentials. Level of persistence in the environment and tendency to bioaccumulate are some of the key factors that make the chemicals of special interest (Rasheed, 2016).

Gas Chromatograph-Mass Spectrometry Analysis

Shimadzu GC mass was used for this purpose; each sample was submitted to GC mass procedures to detect chemical composition. High-dimensional, noisy data are produced by GC-MS; a single sample can contain more than 9 million high-resolution variables. Due to this, certain data processing techniques utilize a variety of preprocessing steps, such as peak detection, spectral deconvolution, baseline correction, and noise filtering. All those preprocessing steps are essential for the identification of VOCs (Skarysz et al., 2018).

Gas Chromatograph-Mass Spectrometry Analysis

Chemical analysis of the samples was done with the Shimadzu GC-MS. The GC-MS procedures identified and quantified the VOCs in the samples. More specifically, it had undergone a series of preprocessing steps, such as peak detection, spectral deconvolution, baseline correction, and noise filtering to interpret the chemical makeup of the samples.

Insilco Tools

Numbers of chemoinformatic and bioinformatic software and databases were used to determine the toxicity of the GC mass chemical of water-polluted samples. TOXTREE (Estimation of Toxic Hazard) software Version 3.1.0, QSAR Toolbox 4.5 Server Application, Discovery Studio Visualizer v2.1.1, Pyrex v1.8 software, Protein Database Bank (PDB), PubChem, and COSMOS databases were used as Insilco tools for the detection of chemical toxicity and its genetic effects.

Method

The GC mass analysis results of chemical compounds were submitted to Insilco for each chemical. The chemical substances must first be identified by their CAS number or by their structure or smile string. TOXTREE software was used to detect the hazard class, which included three classes: low hazard, moderate hazard, and high risk, according to functional groups founded in the chemical composition (Jeliazkova & Jeliazkov, 2011). The procedure used to determine the toxicity of chemical substances was included in the steps below:

1- Chemical identification by using the PubChem and Cosmos databases (Rim, 2020).

2- Level of risk determination by using TOXTREE software (Jeliazkova & Jeliazkov, 2011).

3- Substances submission to the QSARTOOLBOX database in order to find accurate data on their reaction with DNA or protein according to invitro databases for similar substance experiments (Ibrahim et al., 2019).

4- COSMOS databases are used to find the side chains that interact with DNA or proteins (Raunio, 2011a).

5- Data completion of chemical effects on the environment and human health hazards were detected by QSAR toolbox software (Kim et al., 2014).

6- Discovery studio Visualizer v2.1.1 and Pyrex v1.8 software are used to visualize interactions between substances and proteins (Jafary et al., 2021a).

Ligands and protein preparation

With PDB ID 2Z3 T, the cytochrome p450 protein enzyme, which has been the primary target of xenobiotic molecules, was obtained from the PDB database (URL: https://www.rcsb.org). Moreover, the target protein used for the docking study is determined by its X-ray diffraction. Proteins should be in the form of PDB formats. Proteins ought to be stored in PDB format. The 2Z3T cytochrome P450 (CP450) X-ray crystallographic structure was prepared to conduct molecular docking by eliminating any heteroatoms, such as ions, water, etc., by utilizing the Discovery Studio 2021 Client software program. The chain's protein binding sites are chosen, while others are eliminated.

Table 1. Measurement Devices and Methods Used for Chemical Analysis

Chemical Compound	CAS Number	Measurement Device/Method
Light aromatic naphtha	64742-95-6	GC-MS
Heavy aromatic naphtha	64742-94-5	GC-MS
1,2,4-Trimethylbenzene	95-63-6	GC-MS
Para-Nonylphenol	84852-15-3	GC-MS
1,3,5-Trimethylbenzene	108-67-8	GC-MS
1,2,3-Trimethylbenzene	526-73-8	GC-MS
Naphthalene	91-20-3	GC-MS
Cumene	98-82-8	GC-MS
Ortho-Nonylphenol	91672-41-2	GC-MS
Benzopyrene	50-32-8	GC-MS

Molecular Docking

To emphasize the structural conformation of this protein target specificity, structural complexes of the target protein, 2Z3T, with the ligand molecule, Naphtha, were examined using an in-silico method for ligand and receptor docking studies. The PyRx virtual screening tool, which contributes to greater docking accuracy in the current research, integrates Vina and Auto Dock 4.2 with the Lamarckian genetic algorithm as the scoring function (Upreti et al., 2021). The resolution of 3.00 Å was achieved in X-ray crystallography to determine the chemical structure of the macromolecule. Through employing the Pyrex tools to remove bound ligands and water molecules, the study proceeds with the target preparation. Additionally, every chain in this study was eliminated except for Chain A, which only demonstrates a chain with a ligand-binding site. Subsequently, by labeling it as a macromolecule in the PyRx workflow, it is introduced to the PyRx tool. This method applied auto-docking tools to transform the protein and ligand molecules into their appropriate, readable file format (PDBQT). Blind docking was employed for all docking studies, with dimensions of X = 57.862, Y = 62.436, and Z = 56.595 to dock all ligands, and an 8 maximum exhaustiveness was computed for each ligand. The docking studies encompassed all potential ligand-receptor complexes. Every other software parameter was left at its default setting, and all of the bonds in the ligand were free to rotate because the receptor appeared rigid. Using Discovery Studio Visualizer 3.0, the docked structure's final visualization was performed (Jafary et al., 2021b).

RESULTS AND DISCUSSION

GCmass Results

A total of ten compounds were detected by GC mass, as shown in Table 2. The high percentage of these chemical compounds was the aromatic naphtha, which composed between 10 and 20% of the outlet chemical composition as waste water at the southern Baghdad power station. The result agrees with what Oleiwi and Al-Dabbas (2022) in their study showed about this ingredient. The lower percentage included ortho-nonylphenol with 0.1–1%, which is compatible with Porter and Hayden (2010), who showed that the concentration of nonylphenol in the environment is decreasing.

Every identified chemical compound is recognized by its corresponding CAS Number or Chemical Abstracts Service Number . The CAS Number is a unique numerical identifier that identifies every chemical substance described in open scientific literature, providing a reliable and consistent means for identifying chemical substances independent of the naming conventions that may be discipline- or language-specific.

Insilco Study Results

Estimation of Chemical Compound Hazard by TOXTREE

Each chemical compound detected by GC mass for the samples of wastewater from the

Table 2. composition /information on ingredients

Ingredient Name	Percentage %	CAS Number
Light aromatic naphtha	10-20	64742-95-6
Heavy aromatic naphtha	10-20	64742-94-5
124-trimethylbenzene	10-20	95-63-6
Para-Nonylphenol	5-10	84852-15-3
1,3,5-trimethylbenzene	1-5	108-67-8
1,2,3-trimethylbenzene	1-5	526-73-8
Naphthalene	1-5	91-20-3
Cumene	0.1-1	98-82-8
Ortho-Nonylphenol	0.1-1	91672-41-2

southern Baghdad power station that was discharged into the Tigris River was analyzed by Insilco approaches. The TOXTREE software estimated the degree of hazard class for each compound as shown in Table 2. Three compounds were estimated as high class hazards, which included light aromatic naphtha (CAS No. 64742-95-6), heavy aromatic naphtha (CAS No. 64742-95-5), and naphthalene (CAS No. 91-20-3). This estimation agrees with Jia and Batterman (2010).

TOXTREE explanations for high toxicity refer to the presence of aromatic rings (Ratnayaka & Michael Johnson, 2009). The estimation of the remaining chemical compounds in the composition was described by TOXTREE software as low-class toxicity. The explanation, according to TOXTREE, was the ability to substitute aromatic rings in chemical compounds (Mąkosza, 2020). Therefore, the results showed that the most chemical had an aromatic ring, but the chemical with a substitution ring, which was founded in chemicals, was described as low class according to the explanation of TOXTREE (Mąkosza, 2020).

QSARToolbox Databases to Determined Chemical Composition

A total of six functional modules were founded in the QSAR databases, as seen in Figure 1. They included the input module, data module, profile module, category definition, data gap filling, and report module (Ibrahim et al., 2019). A total of ten chemicals were submitted to the QSAR toolbox databases in order to detect all the information for these chemical compounds.

QSAR Databases of Light Aromatic Naphtha (Non-Testing Methods)

The non-testing method in QSAR included all in-vivo and in-vitro studies that were done to experiment with different types of chemical compounds. QSAR databases showed three experiment studies. Human Health Hazards, Acute Toxicity, and LD50 on rats to detect the L50 toxicity of light aromatic naphtha (Figure 2), "Datapoints," the study showed. Rats were utilized to evaluate the acute toxicity of F-64-01 at a dose of 5000 mg/kg of fasting body weight through oral gavage. Following dosing, observations were made every hour for the first four hours and twice a day (a.m. and p.m.) for the following fourteen days. Animals died during the observational period, but loose stools and incoordination were the only clinical effects seen, and they were observed only on the day after the dosing. The LD50 for F-64-01 in rats is >5000 mg/kg. F-64-01 is not categorized as an acute oral toxicant under Regulation (EC) 1272/2008 on the classification, labeling, and packaging of substances and mixtures (CLP) or under Directives 1999/45/EC for preparations and 67/518/EEC for dangerous compounds, based on the characteristics of this study. Also, QSAR databases showed 14 positive studies of irritation tests with erythema infection, as shown in Table 3. The results showed a high toxicity hazard of hydrocarbon naphtha. That agrees with Ratnayaka and Michael Johnson (2009).

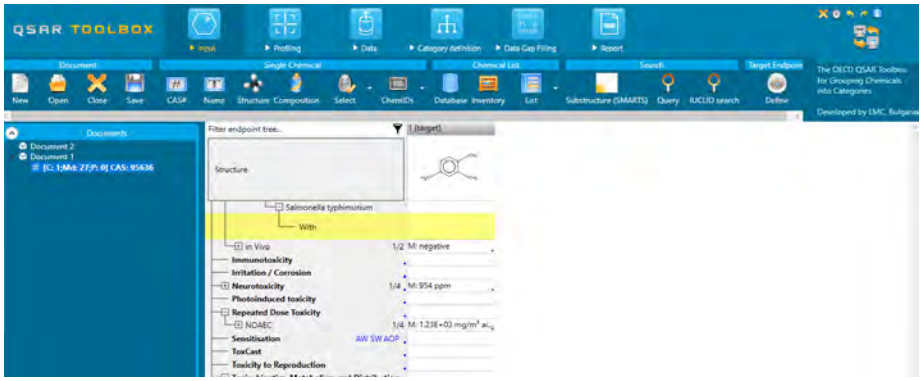

Fig. 1. View of QSAR software showed functional modules

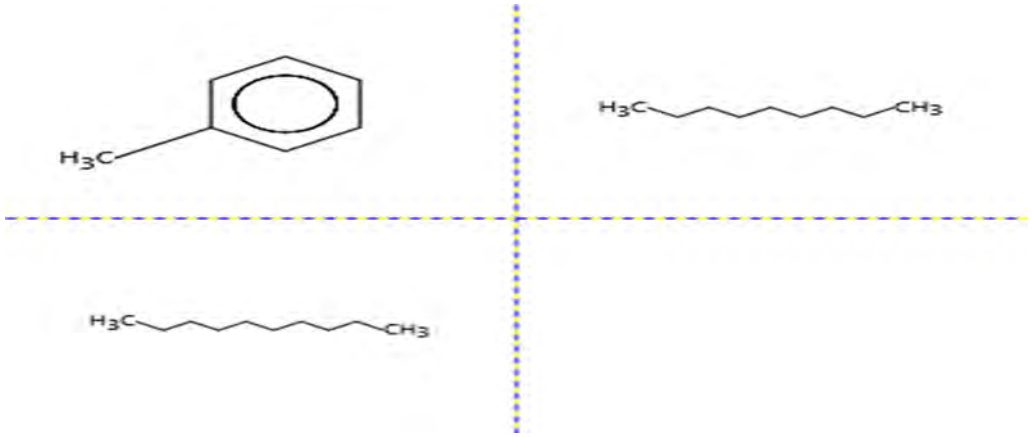

Fig. 2. light aromatic naphtha (QSAR)

Table. 2. TOXTREE estimation of chemical composition of wastewater of power station

Ingredient name	CAS number	Smile string	TOXTREE hazard class	Qsar AMES TEST
Light aromatic naphtha	64742-95-6	c1ccc2ccccc2c1	High class	Positive
Heavy aromatic naphtha	64742-94-5	c1ccc2ccccc2c1	High class	Positive
124-trimethylbenzene	95-63-6	Cc1ccc(C)c(C)c1	Low class	Negative
Para-Nonyylphenol	84852-15-3	CC(C)CC(C)CC(C)c1ccc(O)cc1	Low class	Negative
1,3,5-trimethylbenzene	108-67-8	Cc1cc(C)cc(C)c1	Low class	Negative
1,2,3-trimethylbenzene	526-73-8	Cc1cccc(C)c1C	Low class	Negative
Naphthalene	91-20-3	c1ccc2ccccc2c1	High class	Positive
Cumene	98-82-8	CC(C)c1ccccc1	Low class	Negative
Ortho-Nonylphenol	91672-41-2	CCCCC(CCC)Cc1ccccc1O	Low class	Negative

Table 3. QSAR databases studies of light aromatic naphtha

Test	No. of Test	results
Human health hazard LD 50, rat animal	3	positive
Irritation, Skin, in vivo	14	positive
Ames test	3similarity structure	Positive

Heavy Aromatic Naphtha by QSAR Database

Heavy aromatic naphtha was submitted to QSAR databases by using CAS No. The results showed a complex composition of hydrocarbons, as shown in Figure 3. The results of the QSAR databases are shown in Table 4. A number of studies belonging to aromatic heavy naphtha databases were mentioned in the QSAR database. The studies summarized that a single dosage of the undiluted test compound at a dose of 5000 mg/kg was given to a group of five male and five female rats to evaluate the acute oral toxicity of naphtha (CAS 68516-20-1). Adverse clinical symptoms, such as tremors, ataxia, moribundness, lethargy, abnormal stool, stained coat, alopecia, and hunched posture, have been observed along with the death of one of the animals. The Ames test showed a positive result, which indicated that chemicals have the capability to cause gene mutations and subsequently cancer (Beillard et al., 2003).

A number of complex combinations of heavy aromatic naphtha were detected by utilizing the databases of QSAR. The results are shown in Table 5. The complex combination of heavy

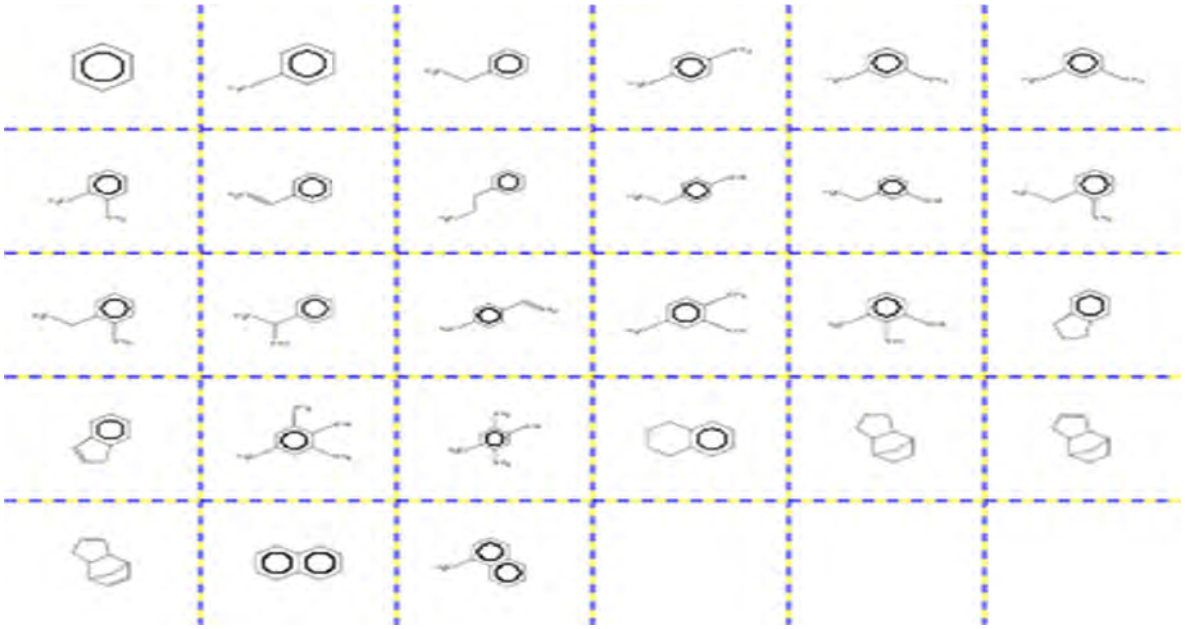

Fig. 3. complex combination of heavy aromatic hydrocarbons detected by QSAR

Table 4. QSAR databases studies of heavy aromatic naphtha

Test	No. of Test	Results
Human health hazard	25	positive
LD 50, rat animal		
carcinogenicity	14	positive
Irritation	43	positive
Ames test	28 similarity structure	Positive

Table 5. QSAR database of heavy naphtha complex combinations obtained from southern power station samples

CAS	NAME	Smiles	BOD	Ames
60-34-4	METHYLHYDRAZINE	CNN	0.2	Positive
50-29-3	DICHLORO_DIPHENYL_TRICHLOROETHANE	C(Cl)(Cl)(Cl)C(c1ccc(Cl)cc1)c1ccc(Cl)cc1	7.1	Negative
50-32-8	BENZOPYRENE;3,4-";_BENZOPYRENE;3,4-	c12c3c4c(c5c(cc4ccc3ccc1)cccc5)cc2	60.7	Negative
50-33-9	PHENYLBUTAZONE	C1(=O)C(CCCC)C(=O)N(c2ccccc2)N1c1ccccc1	0.09	Negative
148-82-3	MELPHALAN	C(=O)(O)C(N)Cc1ccc(N(CCCl)CCCl)cc1	5	Positive
154-93-8	carmustine	C(=O)(N(CCCl)N=O)NCCCl	0.09	Positive
61785-57-7	Benzofurazan,_4-(1-aziridinyl)-7-nitro-,_3-oxide	C1(N(=O)=O)C2C(C(N3CC3)=CC=1)=N(=O)ON=2	1.63	Positive
62-75-9	N-NITROSODIMETHYLAMINE	CN(C)N=O	80	Positive
91-59-8	2-NAPHTHYLAMINE	c12c(cc(N)cc1)cccc2	25	Positive
96-09-3	STYRENE_OXIDE	c1(C2CO2)ccccc1	0.01	Positive
107-13-1	2-propenenitrile	C(#N)C=C	2	Positive
51-79-6	URETHANE	C(N)(=O)OCC	1.8	Negative
53-96-3	2-ACETYLAMINOFLUORENE	c12-c3c(cc(NC(C)=O)cc3)Cc1cccc2	6.2	Negative
54-11-5	Pyridine,_3-(1-methyl-2-pyrrolidinyl)-,_(S)-	c1(C2CCCN2C)cccnc1	75	Negative
54-42-2	idoxuridine	C1(=O)C(I)=CN(C2CC(O)C(CO)O2)C(=O)N1	0.09	Negative
55-38-9	FENTHION	c1(SC)c(C)cc(OP(=S)(OC)OC)cc1	1.8	Negative
55-48-1	atropine_sulphate	C(=O)(C(c1ccccc1)CO)OC1CC2CCC(C1)N{+}2(C).O{-}S(=O)(=O)O	4.5	Negative

aromatic naphtha appeared to have the highest toxicity of most complex combinations. The results showed Ames test positive for the most combinations, so that agrees with Ratnayaka and Michael Johnson (2009) and Vijay et al. (2018). The QSAR database also detected BOD for chemicals in complex combinations. BOD provides a measure of the impact of waste (water) on the oxygen content of a receiving body of water. The number of two combinations showed a high BOD, which included Benzopyrene (3,4) and n-nitroso dimethylamine. Elevated levels of these BOD chemicals have a high impact on the environment and human health, which agrees with Porter and Hayden (2012).

Molecular Docking

Naphtha was docking with cytochrome p450; the cytochrome is responsible for removing a vast number of foreign molecules (xenobiotics) from the body (Ibrahim et al., 2019). The results showed a high affinity between cytochrome chain A receptor and ligand (naphtha) with a binding energy of 6.6, as shown in figure 4. It is clearly evident that the low binding energy indicates a high affinity between receptor and ligand (Bharath et al., 2020).

A number of amino acids were bonded with naphtha by different types of bonds, such as

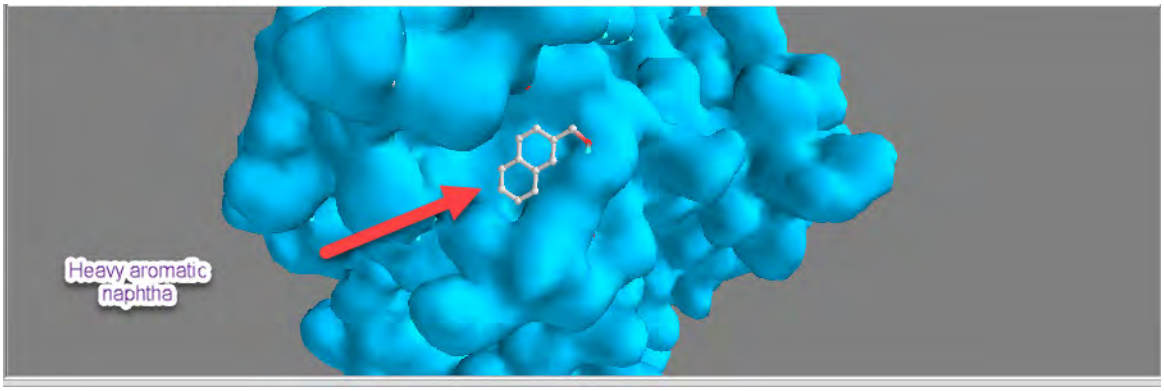

Fig. 4. Binding of naphtha to CYP450 with high affinity due to low binding energy (-6.6), done by PYRX software

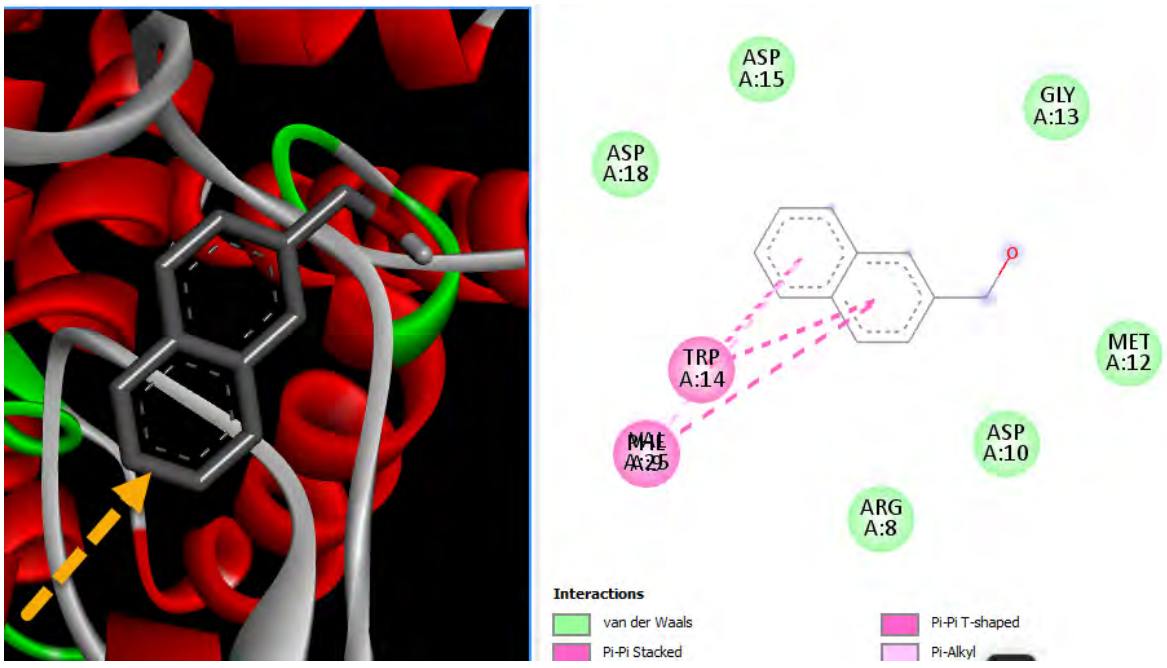

Fig. 5. Interaction between Cytochromes p450 enzyme and naphtha compound as a ligand

Table 6. Types of amino acids, location within chain A of cytochrome p450 and bonds types

Amino acids bonded with Naphtha	Amino acid location within A chain	Bonds type
Asparagine	18	Van der Waals
Asparagine	10	Van der Waals
Glycine	13	Van der Waals
Arginine	8	Van der Waals
Asparagine	15	Van der Waals
Methionine	12	Van der Waals
Tryptophan	14	PI-PI -T-shaped
valine	25	Pi-pi Stacked
Methionine	29	Pi-Alkyl

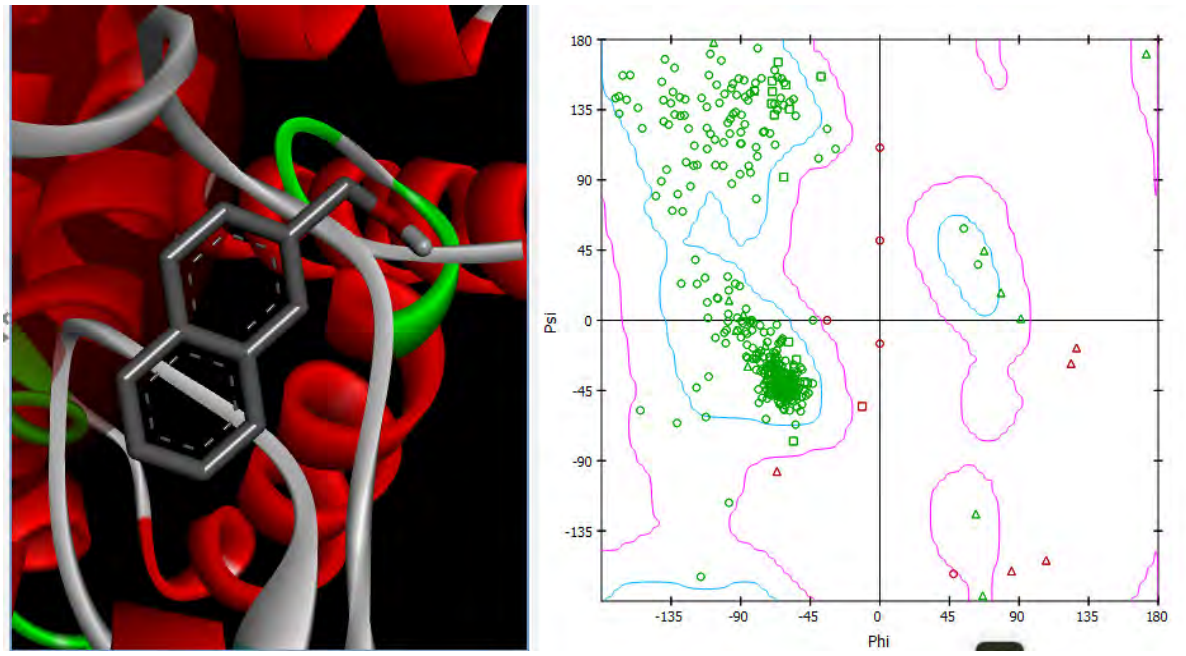

Fig. 6. The Ramachandran plots of chain A of cytochrome p450 functional group distribution

Vander Waals and alkyl bonds, as noticed in figure 5. Asparagine amino acid was the most amino acid that bonded to the naphtha compound, as shown in Table 6. This amino acid contains a polar uncharged side chain; the side chain amide oxygens of asparagine occupy axial hydrogen bonding positions in the plane of the active site group of the cytochrome p450 enzyme (Armstrong et al., 2016).

Many functional groups in chemical structures are known to be associated with the formation of reactive metabolites, which are very often catalyzed by the CYP enzymes (Raunio, 2011b).

The Ramachandran plots showed the distribution of amino acids during the docking process between cytochrome p450 and chemical ligands, as shown in figure 6. The distribution of functional groups indicates that for the cytochrome enzyme, there are 91.6% (470), 6.8% (35), 1.0% (5), and 0.8% (4) residues within the most favored areas, also allowed areas, generously allowed areas, and disallowed areas, respectively. It appears that no more than 2% of residues should be found in the allowed area, and no residue should reside in the disallowed areas, which agrees closely with Agnihotry and Hussain (2020).

The hydrophobicity of amino acids through docking showed high values, as shown in figure 7. Peaks in the profile are equal to the topical maximum in hydrophobicity and decline to

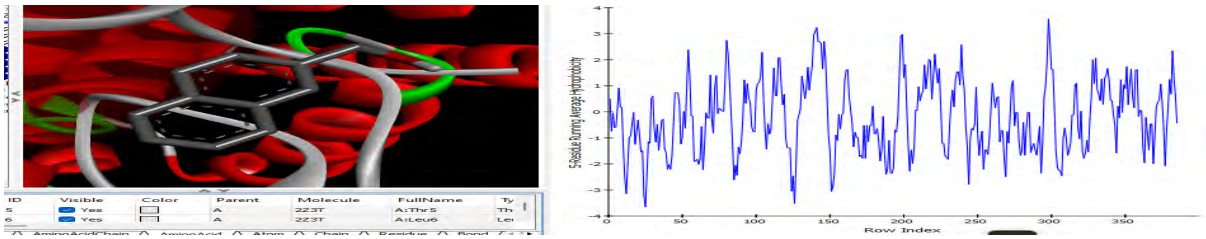

Fig. 7. Hydrophobicity of amino acids through docking

local minima. Variation is dependent on the concept that polar regions along the chain will be disposed better than the residue in the interior, which constitutes a hydrophobic center of molecules, while polar regions (i.e., the decline region in the profile) will be disposed of in the outer region and correspond to chain turns.

CONCLUSION

1- The waste water discharged from the southern Baghdad power station contains a carcinogen compound

2- The molecular docking showed high affinity between Cytochrome P450 and Naphtha with low binding energy.

3- Heavy aromatic naphtha contains complex combinations of aromatic derivatives most of them were showed carcinogenicity.

RECOMMENDATION

1-Add new treatment unit to the electricity power station in order to remove aromatic compounds before they are discharged into the river.

2- Using QSAR software in order to continually check outlet discharges of waste water.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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