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Bioaccumulation of Different Organic Micropollutants in Fishes and its Toxicological and Stress Impacts: A Review

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Abstract: In the past three decades biological and chemical pollutants have become a serious environmental issue posing major threat to the society, industries and public sectors. Toxic contaminants are produced in most household, agricultural and industrial activities. In the past few years different advanced electrochemical oxidation technologies and low-carbon technologies are being used widely for preventing environmental pollution and remediation of the micropollutants particularly in waterbodies. In these technologies powerful oxidizing agents like hydroxyl radicals are formed electrochemically which degrade organic micropollutants till their mineralization. Fish serves as an effective bioindicator of aquatic health due to their higher trophic position in aquatic food chain and high sensitivity to pollutants. As fishes are consumed by humans globally as a major source of protein, it is also used to indicate the impact of aquatic pollution on human health. In this review we discuss the impact of micropollutants on the fish physiology and the advanced wastewater management techniques used for rapid removal of these pollutants from aquatic ecosystem. Here we discuss both advantages and disadvantages of different commonly used wastewater management techniques which would be beneficial to determine which technology would best suit one's specific requirements without causing much harm to the environment, a step towards green and sustainable future.
Keywords: Micropollutants, Organic micropollutants, Fish, bioindicator, Wastewater management technologies, Constructed wetlands

I. INTRODUCTION

In the 21st century, there is extensive use of industrial chemicals in furniture and gadget making industries in order to acquire an improved functionality and long life-span of these equipment used in day-to-day life. Also, to protect crops and for their higher yields pesticides and insecticides are used widely to meet the market requirements. Chemicals are essentials in drug industries and in personal care items for ascertaining proper human and animal healthcare and improved human lifestyle [1–4]. Thus, these chemicals can be released into the environment during manufacture, use, disposal, and sometime accidentally [5–7]. The 1998 Aarhus Protocol (amended in 2009) on Persistent Organic Pollutants (POPs) had designated certain POPs [aldrin, chlordane, chlordecone, dieldrin, endrin, hexabromobiphenyl, mirex ,toxaphene, Dichlorodiphenyltrichloroethane (DDT), heptachlor, polychlorinated biphenyls(PCBs), hexachlorobenzene, dioxins, furans, polycyclic aromatic hydrocarbons (PAHs) ,hexachlorobenzene (HCB), hexachlorobutadiene, octabromodiphenyl ether, pentachlorobenzene, pentabromodiphenyl ether, perfluorooctane sulfonates, polychlorinated naphthalenes and short-chain chlorinated paraffins] to have high bioaccumulation potential causing toxicological impacts to both human beings and animals. Hence the protocol suggested either banning the production and use of some of these chemicals or reduce their atmospheric emission[8,9]. So, it is of utmost importance to mitigate their use in order to reduce environmental pollution. Every year, numerous new chemicals are introduced into the commercial market, and monitoring each one of them becomes a challenging task ,especially those that do not have a CAS number and are unregistered under the regulation named Registration, Evaluation, Authorization and restriction of Chemicals (REACH) of European chemical agency,2007**.** Wastewater Treatment Plants (WWTP) are one of the most important sources of these pollutants via which these chemicals are released [10], followed by industrial releases[11] and runoffs from agricultural lands [12].

These pollutants ultimately can lead to surface water contamination and different aquatic matrix accumulation [13]. Various chemical toxicants mix to form a cocktail of environmental contaminants that might pose an increasing threat to humans and animals compared to an individual chemical contaminant[14,15].

Micropollutants, like certain pharmaceutical compounds and products used to maintain personal hygiene, have been detected in surface water, groundwater, wastewater plants, and natural water. Still, many of these micropollutants are not included in the priority list of water policy as it requires reliable source of scientific evidence regarding their toxicological potentials. Pharmaceuticals , the second most largest group of organic micropollutants mostly fall outside these environmental laws and policies[16–18]. Over 1000 prescribed pharmaceuticals and over 300 drugs requiring no prescription are in wide use and USA and might be released into the atmosphere during their production or use [19]. Also, micropollutants are prioritized in countries like the UK and USA based on individual research. Apart from limitations in instrumentations, the problem lies in identifying and quantifying these large numbers of micropollutants from a wide range of sources. The techniques for identifying and analysing micropollutants in nature are quite expensive and most research emphasizes on the pre-selected particular analytes but not on all the micropollutants found in all natural matrices [17].

The ecotoxicological threat of organic micropollutants is relatively a recent challenge for the world. However, increasing emphasis is given globally to understanding their fate[20]. If released into the environment continuously without regulation, they can pose environmental threat even in low concentration. They are bioactive even in low amounts and can cause detrimental effects on the environment by impairing the ecosystem's functionality and can also be severe to living organisms [21,22].

The impact of anthropogenic activities requires proper monitoring tools to help in the detection and depiction of the physical, chemical, and biological deterioration of the aquatic ecosystem. For detecting the load of contaminants in water, biota like fish, insects, frogs, molluscs, and plants prove to be potential bioindicators[23]. Fishes are more sensitive to different toxicants than other organisms and can be used conveniently for a wide range of toxicological assays to determine the health of aquatic ecosystem[24,25]. Fishes are the most diverse class of vertebrates in the aquatic ecosystem that make them an appropriate model to study aquatic toxicology[26] .Fishes are placed at the endpoint of the aquatic food chain and hence they bioaccumulate pollutants like heavy metals and pesticides to a greater extent and pass them to their consumers, including human beings, causing severe chronic diseases in humans. Fishes serve as an appropriate indicator of the ecological status of rivers as they have particular habitat requirements and shift habitat during different life stages like larva, juvenile, or adult [27]. They indicate the trophic status of the aquatic ecosystem as they are both primary and secondary consumers of the aquatic food chain[28]. As fish meat has high protein and mineral content, cardioprotective compounds, and low-fat content, it is considered a nutritious food. Fish muscle acts as the deposition site of organic micropollutants(PCBs, dioxins, PAHs, etc.), trace metals, and pesticides that threaten global food safety[29]. Consuming heavy metal contaminated fishes can severely impact human health. For instance, from 1953 to 1960, thousands of Japanese died consuming mercury contaminated fishes. Hence in the last few decades, there has been a global concern regarding presence of contaminants in seafood and freshwater and marine fishes [27].

The main challenge of the ecotoxicological assays is to provide a detailed scenario of additive, combined, and antagonistic impacts of mixtures of chemical contaminants from different sources of pollution on the biota [30].Ecotoxicological tests are usually used to assess the impact of pollutants at both individual level, and how that can translate into population level, and their ecological implications [31].Parameters like reproductive ability, rates of survival or mortality, and development are important to study the impacts of ecotoxicology on the entire population rather than on an individual level[32]. Mathematical models are often used to predict toxicological impact at a population level based on the test results obtained at an individual level. Using the test results of the bioassays, these models can be used to simulate the effects of environmental contaminants in populations. For a better understanding of the impacts of the toxicants on the fish embryo, biochemical biomarkers play an important role. With the help of biomarkers, it is possible to detect toxicity at an early developmental stage of organisms exposed to xenobiotics and hence are regarded as a sublethal indicator of environmental pollution[33].

II. FISH AS BIOINDICATOR SPECIES OF AQUATIC POLLUTION

Sub-lethal effects of pollutants vary at various stages of biological organisation like cell, tissue, organ, individual, population, and community. The biomarker approach is used to investigate specific biochemical, physiological, morphological, and cellular reactions to the exposure of pollutants in a particular ecosystem [34].A bioindicator species is an individual or group of organisms representing the condition of the entire ecosystem. There are certain requirements for being a bioindicator with respect to the physical and chemical parameters of the environmental variation. The impact of these environmental variations are prominently marked in the bioindicator species and hence they represent the condition of its residing ecosystem[35]. Bioindicators are also often defined as organisms prominently reacting to the human-caused effects in their environment. Fishes are regarded as useful bioindicators of water quality as they exhibit differential sensitivity towards pollution[36]. The integrity of the aquatic ecosystem can be well indicated by fishes from microhabitat to catchment areas as they have complex requirements of habitats[27].

They are beneficial in describing the natural features of the aquatic ecosystem[37]. Fishes can be used as an efficient biomonitor for evaluating metal load in waterbodies[38].Owing to the average size of fishes and their organs, a wide array of analytical assays can be carried out on them.

Haematological, histological, biochemical, morphometric, genetic, and pathological tests can be appropriately carried out in fishes and thus fish model can be beneficial in medical research[39].Fishes have always been used as an aquatic matrix to determine health of aquatic health as they absorb the chemicals directly from their ambient medium via dietary intake [40]. There are different chemical toxicants that biomagnifies in the aquatic food chain, and fish being top consumer in the aquatic food chain, the distribution and bioaccumulation of those chemicals is most prominently seen in them[41–43]. As bioaccumulation of micropollutants is high in fishes, it poses a severe risk to ,the piscivorous birds, mammals, including human beings .Hence monitoring the fish species is important to assess the level of bioaccumulation of pollutants in waterbodies and their effect on the overall health status of the aquatic ecosystem[44].

Also, fishes are specific bioindicators of various environmental sections in relation to their natural environment and position in the food web.

They show variable rates of bioaccumulation and biotransformation of xenobiotics [45]. The community structure of fishes depends on the aquatic ecosystem under investigation, and it is hard to find the same fish species at every site of study interest. To address this problem, scientists often conduct transplant experiments[46].

III. IMPACT OF ORGANIC MICRO-POLLUTANTS ON THE PHYSIOLOGY OF FISHES

Organic micropollutants are lipophilic and pose an ecological threat, causing endocrine disruptions and congenital disabilities and also negatively impacting the reproductive system[47,48]. Physicochemical parameters of water, like dissolved oxygen and temperature, correlate with the bioavailability of organic contaminants in fish species [49].

Organochlorine pesticides (OCPs) are classes of synthetic pesticides used widely in chemical and agricultural industries. PCBs are widely used in electric capacitors as insulating fluid, in transformers in industries, as lubricating liquid, in hydraulic machines, etc. [50,51]. Both OCPs and PCBs are lipophilic and persistent in the ambient medium, which biomagnify through food chain and ultimately cause genotoxicity, neurotoxicity and toxicity in reproductive system, and even carcinogenesis in the aquatic species even in low concentrations [52,53].

Reports of ECHA (European Chemical Agency) (ECHA, 2017) highlighted the toxicological impacts of PAHs like carcinogenesis, mutagenesis, high bioaccumulation potential, reproductive toxicity, etc.. The report stated that this group of micropollutants pose a severe risk to both environment and human health.

Fluoranthene and pyrene are the PAHs that are abundant from pyrogenic sources. They are very persistent in nature as they are not degraded and remain unaffected under other removal procedures except sedimentation [55]. These micropollutants are indicators of anthropogenic activities like the combustion of fossil fuels, industrial and urban effluents etc.[56]. Owing to their severe toxicological impacts on the environment OCPs and PCBs are restricted from being produced or used on large scale. But in different developing countries like Brazil, on an irregular basis, these chemicals are used and deposited[57]. USEPA - United States Environmental Protection Agency, 2017 reported OCPs and PCBs as priority contaminants with well-recognized toxicological impacts[58].

Table 1 summarizes the impact of different micropollutants on the morphology and physiology of different fish species exposed to variable environmental conditions.

A. Endocrine Disruption in Fishes

Emerging pollutants like pharmaceutical products, caffeine, and UV filters arise from domestic, oil refineries, agricultural and industrial effluents. PAH was detected in fish bile earlier, and an increase in biological oxidative stress and liver damage was found in the study due to PAH accumulation [59].

Fishes, if exposed to PAHs, can have endocrine disruptions as these chemicals alter the steroid levels in fishes. The level of vitellogenin in fishes not only depends on the Endocrine Disrupting Chemicals (EDCs), which directly bind with the estrogen receptors but also on exposure to the chemical pollutants there is the alteration in hormonal levels in the fish body. Drugs can even lead to liver injury in fish[60]. EDCs can also increase and decrease the production of steroid hormones such as estradiol and 11-keto testosterone disrupting the equilibrium in fish physiology[48,61].

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Table 1: Morphological and physiological impact of different micro pollutants on fish species

Pollutants	Fish	Collection site/Type of study	Health impacts	References
PCB 126	Japanese medaka (*Oryzias latipes*)	Lab based dose study	i. Depressed humoral immunity ii. Decreased Antibody Forming Cells (AFC) iii.Induced immunotoxicity	[53]
OCP, PCB and polybrominated diphenyl ethers (PBDEs)	Male tilapia (*Oreochromis niloticus*)	4 reservoirs on Iguaçu River, Southern Brazil	i.. Altered CYP1A causing endocrine disruption ii.Vitellogenin gene induction iii. Incresed No. of eosinophils in testis	[59]
PAHs, OCPs, PCBs, heavymetals, Al,F e and Mn	*Rhamdia quelen*	Iguaçu and Jordão rivers, Southern Brazil	i. Antioxidant system activation (reduced non-protein thiol range) ii.Long lab exposure increased embryo mortality rate iii. Increased SOD activity due to oxidative stress iv.Malformation of spine	[62]
16 PAHs and heavy metals	Larval embryos of *Rhamdia quelen*	Upper Iguaçu River, Southern Brazil	i.. Skeletal defects like lordosis and scoliosis ii.Tail deformities iii.Significant rate of mortality iv.Decreased embryo to larva survival rate v. Defect in spine, cranium and thorax	[63]
Triclosan, triclocarban and their binary mixtures	Embryo of *Rhamdia quelen*	Collected from Panama fish farm (Paulo Lopes, SC, Brazil) followed by lab-based dose study	i.Fin deformities ii.Decreased SOD activity iii. Increased AChE activity	[64]
PAHs,PCBs,OCPs,PBDEs ,phthalates and bisphenol-A	*Salminus brasiliensis*, *Prochilodus lineatus*, *Rhamdia quelen*, and *Pseudoplatystoma corruscans*	AtubaRiver,Southern Brazil	i.High mortality rate and pollutant sensitivity in S. *brasiliensis* and P. *lineatus*. ii. spinal torsions iii.pericardial edema iv. abnormal barbel development of eye pigmentation vi. Cranial deformity	[65]
Pesticide residues	*Danio rerio*(zebrafish) embryo	Vacacaí river, SouthernBrazil	i.Increased level of GSTand thiobarbituric acid reactive substance (TBARS) ii.Delay in hatching rate iii.Decreased heart rate iv.IncreasedAChE	[66]
Benzo[a]pyrene	Zebrafish larvae(*Danio rerio*)	Lab based dose study	i.Increased mortality in high dose ii.Deformed tail iii.Deformed pectoral fin iv.Deformed jaw v.Defect in optic and otic vesicles	[67]
Pyrene	Rockfish (*Sebastiscus marmoratus*) embryo	Lab based dose study	i.Impaired skeletal formation ii. Chondrocyte proliferation disrupted iii.Deformed lower jaw cartilage	[68]
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	Female rainbow trout (*Oncorhynchus mykiss*)	Lab based dietary exposure	i.Reduced survival rate ii.Poor survival of fry iii.Edema and developmental defects in fry	[69]
PAHs and heavy metals	*Aphanius fasciatus*	Tunisian coast	i.Spinal anomalies ii.Skeletal deformities iii.Deformed mandibles iv.Deformed vertebrae and arcs	[70]
Mixture of fipronil and fungicides (pyraclostrobin and methyl- thiophanate)	Adult zebrafish(*Danio rerio*)	Lab based dose study	i.Decreased non-protein thiol ii.Decreased catalase activity iii.Increase in SOD and catalase ratio iii.Locomotor impairments iv.Increased ROS and MDA in hepatic tissue v.Imbalance of antioxidants in brain	[71]
Fludioxonil and triadimefon mixture	Zebrafish (*Danio rerio*)	Lab based dose study	i.Increased GST activity ii. Induced oxidative stress due to increase in ROS, T-GSH and malondialdehyde(MDA) iv.Embryo apoptosis	[72]

B. Teratogenesis and Neurotoxicity

Neurotoxicity is indicated by induced apoptosis and necrosis. Zebrafish serves as an appropriate model to predict teratogenesis and neurotoxicity in mammalian system. Atrazine, and TCDD are specifically recognised as teratogenic compounds in fishes whereas 2,4-dichlorophenoxyacetic acid (2,4-D), dieldrin, non- phenol, dieldrin and nonylphenol are neurotoxic causing catecholaminergic neuron toxicity in fish model[73]. Motor neuron functions like feeding, swimming, escaping from predators are affected by 2,4,-D sublethal exposure[74].It also causes apoptosis of brain cells, disrupted growth of motor neurons and decrease in motility and it is teratogenic to fishes[73]. Atrazine affects production of dopamine, functionality of motor neurons, causes brain cell death and developmental toxicity[75]. DDT increases motility, causes apoptosis of brain cells, loss in catecholaminergic neurons and is highly teratogenic in both medium and high exposure. Dieldrin causes tremors and loss of dopaminergic neurons and is little teratogenic in fishes[73] .Malathion causes significant toxicity at early developmental stages and apoptosis of brain cells (Lien et al., 1997; Cook et al., 2005) .TCDD exposure causes pericardial oedema, retarded growth, decelerated circulation, vascular endothelial cell toxicity, brain cell necrosis and apoptosis, shrinkage of brain, decreased heart rate and motility and is highly teratogenic[78,79].

C. Deformities

Deformities are a major eco-physiological threat for individuals and can have severe adverse effects on the population of fish with respect to fitness, survival rate, and reproductive ability. There can be various morphological defects in fishes exposed to different environmental contaminants [67,80]. Defects in the spine, thorax, and cranium were recorded in *R. quelen* exposed to different type of organic micropollutants [62]. The proliferation of the cartilage cells was disrupted, resulting in skeletal defects in *Sebastiscus marmoratus* exposed to pyrene[68]. TCDD arising as a by-product of different industrial procedures like production of chlorinated insecticides and pesticides, and paper bleaching can lead to deformities of the spine and tail of various fish species [81]. When fishes are exposed to a combination of heavy metals, PAHs, estrogen and other related compounds, they have a higher probability of developing spinal deformities [70].

D. Biological Oxidative Stress

Toxic biochemical effects of environmental contaminants are exerted by the production of Reactive Oxygen Species (ROS) like superoxide radicles. These radicals arise as a by-product of xenobiotic redox cycling or due to the process of biotransformation. Hydroxyl radicle (OH) reacts the most among all other ROS, and its precursor is superoxide radicles [82]. Superoxide dismutase (SOD) is an antioxidant enzyme. It forms the first line of defence in the antioxidant system, acting as catalyst in the dismutation process of superoxide radicals resulting in the formation of hydrogen peroxide (H_2O_2). H_2O_2 on further metabolism resists the formation of OH radicals and thus prevents biological oxidative stress in organisms[83]. Several studies have been conducted on the role of xenobiotics in inducing biological oxidative stress in fish species, showing that exposure to xenobiotics induces increased production of SOD and ROS [72,84]. If the antioxidant system in the body of organisms is highly activated, there can be systemic impacts of toxicology, ultimately leading to chronic diseases.[85]. There is a prominent relationship among biological oxidative stress, skeletal deformities like lordosis and sclerosis, and peroxidation of lipids in fish species. Bone metabolism is significantly dependent on the lipid content in the skeletal tissues in fish, and this explains the spine defects in fishes vulnerable to peroxidation of lipids [86,87].Intracellular toxicants in fishes are eliminated by the activity of glutathione transferase (GST), and it is also responsible for the detoxification of the organic micropollutants in their ambient aquatic environment. Hence, GST can be used as an important biomonitoring tool to assess the health of the aquatic ecosystem [88].

E. Inhibition of Acetylcholinesterase (AChE) activity

AChE activity inhibition is a significant biomarker of carbamate and organophosphate exposure in fishes and can be used for monitoring status of fishery resources in waterbodies. If AChE inhibition is very high in muscles of fishes , then it can cause severe health risks to humans consuming them[89]. An dose based laboratory exposure to organic micropollutants like PCBs, pesticides, and OCPs showed decrease in the AChE activity in fish brain. Inhibition of AChE level can cause the cholinergic nerves to get excessively stimulated, ultimately resulting in lethargy in swimming, convulsions, spasms, and other behavioural changes [66].

F. Effect on Cardiac Development and Heart Rate

Heart rate is a very important parameter in the toxicological embryo tests of fish. Exposure to herbicides and fungicides reduces the heart rate. Any injury in the heart leads to reduced blood transportation to the fish embryo, affecting energy transport and the overall embryo development[66,90].

Organic micropollutant dioxin causes malformation of heart and defect in cardiac function. Fish embryos when exposed to TCDD results in reduced heart rate, decreased cardiac output and regurgitation of flow of blood at the atrioventricular valve[91]. Carbamate causes bradycardia and heart malformation in fish embryo[92,93].Nicotine exposure causes looped heart and pericardial oedema in fish embryo [94].

IV. WASTEWATER TREATMENT LEGISLATIONS AND GLOBAL WASTEWATER MANAGEMENT STRATEGIES

The European Green Deal [95] aimed at a toxicant free environment with no environmental pollution and its broader goal is the protection of the health of both humans and the ecosystem. The European Green Deal aims to avoid the detrimental effects of chemical contaminants, including pharmaceutical toxicants, in air, water, or sediment. The Farm to Fork strategy [96] which has been adopted in the recent past, aims at the reduction of total EU sales of antimicrobial compounds used in animal farms and aquaculture farms by 50% (by the year 2030), ultimately cutting on this particular source of environmental pollution. Different other strategies and initiatives have been taken up throughout the globe with a common aim to reduce environmental pollution, which includes the 8th Environment Action Programme [97], the Biodiversity strategy [98], the Sustainability related chemical strategy[99], Circular economy action plan [100], etc. The environmental protection strategies emphasize the wholesome production and usage of natural resources, raw materials and chemicals which are considered as safe and sustainable and create negligible negative impacts on the environment. Transdisciplinary approaches are adopted all over the globe for improvising wastewater treatment techniques in Sewage Treatment Works (STW). European Union Strategic Approach was adopted in March 2019 by the European Commission to control pharmaceutical compounds in the environment. This approach addresses the environmental effects of all lifecycle stages of pharmaceutical products in both animals and humans including their designing, production, usage and disposal [101]. The Water Framework Directive 2000/60/EC is aimed at defining and prioritizing the environmental contaminants with high risk potential (Directive 2000/60/EC,2000). Directive 2000/60/EC was further improvised by formulating Directive2008/105/EU (defined 33 environmental contaminants and standardized their environmental quality and created watchlist for 10 compounds),Directive 2013/39/EU(incorporated 17-alpha-ethinylestradiol, diclofenac, 17-beta – estradiol in the watchlist), Directive 2015/495/EU(added antibiotics namely erythromycin, azithromycin, estrone and clarithromycin to the watchlist), Directive 2018/840/EU(removed diclofenac and added ciprofloxacin and amoxicillin to the list) and finally Directive 2020/1161/EU (removed 17-alpha-ethinylestradiol, 17-beta-estradiol estrone and other antibiotics from the list except amoxicillin and ciprofloxacin)[103–106].European Union(Strategic Approach to Pharmaceuticals in the Environment and European One Health Action Plan against Antimicrobial Resistance) following Decision 2020/1161/EU decided to incorporate certain sulphonamide antibiotic(sulfamethoxazole), diaminopyrimidine antibiotic (trimethoprim) and the antidepressant drug (venlafaxine), O-desmethylvenlafaxine and ten pharmaceutical compounds of azole class into the watchlist (EU water legislation December 2020). Urban Wastewater Treatment Directive (2019) stated that this policy has helped in the reduction of pollutant load in water and hence has improved the overall water quality [108]. This 2019 directive has also raised concern regarding the emerging micropollutants(pharmaceutical products and microplastics), total energy expenditure in a wastewater treatment and associated management practices of sludge [109]. To address these and to reach UN Sustainable Development Goals there should be a revised and improvised version of the Urban Wastewater Treatment Directive in the upcoming years[110].

V. ORGANIC MICROPOLLUTANT REMOVAL TECHNIQUES

Depending on the origin, waste water contains a complex mixture of organic and inorganic pollutants and if they are discharged into the environment without treatment, it creates huge adverse impact on the ecosystem. Based on the characteristics of waste water, different waste water treatment plants (WWTPs) have been selected to remove micro-pollutants such as pharmaceutical residues, personal care products, various household chemicals, and biocides/pesticides from the wastewater. But the chemicals which are used for these treatments may react to form new products which pose severe impact on the environment. Hence, eco-friendly techniques are urgently required for wastewater treatment which would cause negligible negative impact on the environment [18]. The advantages and disadvantages of major wastewater management technologies is depicted in **Table 2**. Hence there is a need for nature-based technologies with lower carbon emissions. New nature-based technologies like Constructed Wetlands(CWs), which is a plant-based processes having budget-friendly operational and maintenance cost and low energy requirement, are being sought after these days[111]. Anaerobic membrane bio reactors (AnMBR) and enzyme based technologies are eco-friendly technologies for wastewater treatments and efficiently remove pharmaceuticals, high strength organic contaminants in wastewater like dairy, sugar, and alcoholic products[112].

AnMBR is an integrated approach of anaerobic bioreactor and microfiltration techniques which has low energy demand and efficiently produces biogas and maintains the system's energy neutrality [113].Only partial removal of micro pollutants and other emerging pollutants of global concern occur if only biological treatment is applied in wastewater. Hence oxidant or adsorbent techniques are highly suggested in water industries like activated carbon and ozonation [114]. Present lifecycle assessment framework studies for assessing the net environmental effectiveness of ozonation and granular activated carbon (GAC) filters for removing micro-pollutants have shown proven values of pollutant removal efficiency and toxicological potentials. The results from these studies further concluded that the advantages of these innovative wastewater treatments were outweighed by increased direct effects and energy and resource requirements[115].

A. Ozonation

Nowadays, advanced oxidation techniques are being used to reduce the concentration of micro-pollutants in wastewater, and among them, ozonation is one of the best processes. In this process, oxidation of organic micro-pollutants present in wastewater occurs directly or through the formation of different OH radicles [116]. On-site synthetic air is used in large-scale wastewater management plants to generate O_3, which is then scattered into oxidation reactors at 4-15mg/lit wastewater concentration. 5–60 g CO_2-equiv./m^3 is the estimated carbon footprint of the ozonation process, depending on the sources of energy and operating conditions. The chemical reactions involved in the process of ozonation are usually non-selective. However, different studies showed that the reduction degree of electron-rich complexes is much higher than other compounds. Pharmaceutical products like diclofenac, sotalol, gemfibrozil and other organic micro-pollutants from the wastewater can be removed effectively by O_3 [108]. Though O_3 has very high reactivity, ozonation of wastewater does not result in the complete oxidation of the organic compounds to carbon-dioxide and water. There are chances of the production of transformation by-products that can be even more detrimental than the original toxicant [117]. It becomes hard to predict the ecotoxicological net result of ozonation technique in municipal wastewater management as the degradation of toxicants and production of detrimental by-products occur collaterally. Oxidation by-products that are generally unknown have potential toxicity in the ecosystem, like bromate formation in bromide rich wastewater. For better results, it is suggested that ozonation is followed by other biological treatments like sand filtration or adsorption techniques like GAC in order to remove the toxic transformation by-products[118]. The demerits of wastewater ozonation technique is its high operating and maintenance costs, poor reduction of COD and BOD and requirement of complex equipment and systems for running[119]. It is suggested to apply food-choice trials to understand the concept of ozonation in wastewater treatment[120]. Moreover, it complements currently used toxicological impact assessing tools in ecotoxicology studies.

B. Activated Carbon

Activated carbon which is formed from carbonaceous materials like coal or husk of coconuts, is widely used to eliminate micro-pollutants from wastewater. Activated carbon acts as an effective adsorption element after being activated chemically or thermally. The inner surface area of activated carbon measures 800–1800 m^2/g and possesses hydrophobic surface features [121]. The toxicants in the wastewater, which are non-polar or have a positive charge are effectively removed by the activated carbon technique. These compounds include metoprolol, ibuprofen, atrazine etc. [108]. In advanced sewage treatment works (STW) conducted by the municipal corporations, activated carbon is used either in the powdered form, i.e., Powdered Activated Carbon (PAC), or in granule form, i.e., GAC. The particle size of PAC ranges between 50–100 µm, whereas for GAC, it is 0.5–4 mm. 10-20mg/lit is the concentration of PAC dose in wastewater treatment. After, the activated carbon adsorption, it is separated from the water body and is burnt together with the wastewater sludge. However, a continuous application is required for GAC in a fluidized bed reactor or in a fixed reactor. GAC doesn't have longer adsorption capability after use; therefore, it is thermally regenerated and then partially used. As both PAC and GAC require only pumping or stirring, the on-site energy requirement for these processes is low. The highest amount of carbon is emitted during the production of activated carbon elements via the processes like extraction of raw materials, combustion, and carbon activation [122]. Carbon footprints used in this process are dependent on the utilization of raw materials. If coconut husk is used as the raw material, it ranges between 5 g CO2-eq per kg PAC; if coal or lignite is used as raw material, the same varies between 18 g CO2-eq per kg PAC [123].The carbon footprint of GAC is lower than that of PAC as in the GAC process, 90% of the already utilised adsorbent can be renewed and reused. The carbon intensity of the activated carbon treatment largely depends on the PAC doses and the bed life of GAC, respectively. As per (European Commission, 2019) to produce goods required in construction industries like basins, pipes etc., 19-27% of total carbon footprint is required and depends on the particular design of the plant.

Table 2: Advantages and disadvantages of common wastewater treatment practices along with working principle

Wastewater treatment methods	Mechanism of action	Pros	Cons	References
Ozonation	Wastewater oxidation using O_3. OH radicals formed from O_3 and degrade the micro-pollutants.	i. Negligible harmful wastes are generated ii. Requires only 15-20 mins of contact between ozone gas and wastewater iii. On-site generation of ozone, no danger associated with storage iv. Ozone eliminates colour and smell effectively	i.Half-life is very short, only 20 mins ii. Produces harmful by-products. For, eg. It produces bromate from waste water rich in bromide iii. Reduction of high BOD and COD is not very efficient iv. Operation and maintenance are expensive v. For proper functioning requires complex instruments and a control unit vi. O_3 can't impact on salinity vii. High dose of O_3 is required to resistant dyes	[119,124,125]
Hydrogen peroxide	Direct oxidation of wastewater components and thus reducing BOD, COD, or TOC	i. Does not produce residual gas ii.H_2O_2 is a strong oxidizer iii. It doesn't have a strong smell and isn't an irritant iv. No sludge formation	i. Requires high dose as it has deficient anti-microbial activity.	[125,126]
Fenton process	Oxidating using H_2O_2.Fe(II) majorly; 4 steps namely oxidation, neutralization, flocculation and sedimentation	i.No additional energy requirement for process activation ii. Cost- effective and easy to manage the process iii.Material transfer isn't restricted; the process is homogenously catalytic iv. No need of catalysts	i. Process is efficient only in a narrow range of pH ii. Can produce new contaminants by means of degradation compounds	[127]
Activated carbon	Removes dyes by adsorption	I. Able to remove a wide range of dyes ii. Low operational cost iii.Can be recycled	i. Difficult to regenerate ii. Can be reused partially	[128]
Constructed wetlands	Settlement of suspended particles, filtration, precipitation, adsorption, biotransformation, ion exchange, microbial breakdown and modification of contaminants, uptake by the plants, nutrient transformation by microorganisms and vegetation, and natural degradation of the pathogens	i. Cost-effective ii.Low energy demand Facilitates water recycling iii.Provides habitat for aquatic organisms iv.Boosts tourism, sporting, and aesthetics of accessible open space v.Prevents flood caused by storm water	i.With age, wetlands lose contaminant removal efficiency ii. Requires large land area iii.Less consistent performance than conventional wastewater treatments iv.Can't be fully dried v. Plants can't withstand being submerged	[129,130]

C. Fenton Process

The Fenton process is widely used to remove organic contaminants from wastewater and ensure water safety effectively. Pollutants that are highly stable are efficiently removed by this process [131]. It is also used for disinfecting water [132]. The process showed maximum efficiency at pH 3 and was first time in-use for oxidizing maleic acid [126,127]. It consists of 4 major steps: i. Oxidation ii. Neutralization iii. Flocculation IV. Sedimentation. However, pollutant removal occurs mainly during the oxidation and coagulation steps. The oxidation step includes the formation of coagulants and OH radicals [133]

D. Electrochemical Oxidation Process

It is used in pilot and wastewater treatment plants [134]. This process is used for reduction of harmful effluents into the surface water to resist ecotoxicology in the recipients [135]. Here, anodic oxidation directly produces OH radicals, or these radicals are generated from the Fenton reagent, the second strongest oxidation agent. The OH radicals maintain an optimum reduction potential (E=2.8 Volt) for micro-pollutants [136]. Electrochemical oxidation is applicable in various sewages and can mineralize the micro-pollutants entirely [137]. As the electron flows directly via the anode, the OH radicals can mineralize directly. Anodic electrochemical oxidation can also be used effectively in removing derivatives of drugs [138].

E. Hydrogen Peroxide (h_2o_2) Technique

H_2O_2 effectively removes organic micro-pollutants, especially pharmaceutical compounds, leading to improved OH radical production [139]. UV method of wastewater treatment is often used along with this technique to achieve better mineralization of micro-pollutants [140].

F. Ultrasonic Irradiation

It is a widely used pollutant-free technique to remove micro-pollutants like ibuprofen, ethyl-paraben, methyl-benzotriazole, etc. This technique uses chemical reactions, shock waves, and stress [141]. Ultrasound in 3-D longitudinal waves spreads in this process and the wavefront causes an increase and decrease in pressure after passing via a medium .This pressure fluctuation is mainly dependent on the sound wave intensity. There can be the formation of very tiny cavities as the fluid medium becomes discontinuous due to an excessive increase in pressure. As a result, the microscopic cavities formed are pulsed via the liquid medium resulting in 4000-6000ºC and 300-500 bar temperature and wastewater pressure, respectively. Ultimately, this temperature extremes lead to micro-pollutant degradation [126].

G. Microwave Technology

The microwave technique is a molecular level heating technology and is used both in industries as well as for domestic purpose. Pollutants like azo dyes, different pesticides, and perfluoro-octanoic acid which are hard to degrade, are efficiently removed by potassium persulfate (oxidizing agent) in this technique. Potassium persulfate is used after being irradiated by the microwaves during the oxidation process. When combined with oxidation treatments, microwave technology can effectively degrade the micro-pollutants. The driving force of this technology is temperature and pH. Microwave technology can also be used in combination with the Fenton process. In that case, the efficiency percentage of the process ranges between 40-60% depending on the Fenton dose. It has wide range of advantages, it causes reduction in reaction time, increases reaction's selectivity, requires low activation energy, doesn't require large sized equipment, reduces waste production, reactions are easy to control , increases yield of products and purifies them.[142–144].

H. Catalysis

The catalytic process includes homogenous and heterogeneous catalysis and also bio-catalysis. Homogenous catalysis is very selective and deals with transition metal composites or specific organic elements with one or multiple reactants in a single phase. Heterogeneous catalysts do not mix well with the reactants and forms a different phase. The surface area of these catalysts is large, and the catalysis occurs in the pores. The advantage of the heterogeneous catalysis process is that it is a green eco-friendly technology, can separate the catalyst easily from the products, treats organic wastewater effectively and is more cost-effective than the homogeneous catalysis. However, in many cases, the catalysts do not act selectively, which is its drawback [145,146]. The disadvantage of homogenous catalysis is that its operational cost is very high. Bio-catalysis can be defined as the use of natural substance (biocatalysts) which are proteinaceous compounds working specifically only on the predetermined substrates. Their main benefit is the fact that they are very selective in reaction.

However, their drawback is their high cost and extreme sensitivity to temperature, pH, concentration of product, and ionic strength[147]. Heterogeneous photocatalysis is eco-friendly technology which specifically removes organic micro-pollutants and Advanced Oxidation Processes (AOP) fall under this broad technique. There is wide range of advantages of photocatalysis process in removal of micropollutants from wastewater ,it is runned by a full renewable source of energy or sunlight, can occur under mild temperature and pressure, doesn't cause secondary contamination and is cost effective etc.[148]. In heterogeneous photocatalysis, mainly titanium dioxide (TiO_2) is used as it is chemically and photochemically stable. The only disadvantage of this technique is its high energy requirement ((3.0-3.2 eV) covering the UV electromagnetic spectrum. Diclofenac, ibuprofen, sulfamethoxazole, non-ionic surfactants, plasticizers, insect repellents, fragrances, heavy metals, propranolol, carbamazepine, 17 alpha ethinylestradiol and 17-beta-estradiol are some major micropollutants which are effectively removed by photocatalysis [148,149].

I. Constructed Wetlands

Constructed wetlands are artificially built shallow basins containing gravels, sediments and plants that are tolerant to saturated ambient conditions. Different types of constructed wetlands are used in wastewater treatment such as surface flow wetlands, subsurface flow wetlands and a hybrid of these two types[150]. However, combining these two can also be integrated with other traditional wastewater treatment practices for more effective results [151,152]. Constructed wetland primarily depends on the prevailing environmental situations and their specificity for domestic, agricultural, storm water and coal mine effluent treatments[151]. These wetlands are specifically used for removing pollutant loads from both primary or secondary household sewage and agricultural effluents. They are also used to treat effluents from active and dumped coal mines [153]. Currently, constructed wetlands are utilized to prevent floods in urban areas caused by storm water and this approach is being accepted globally [130]. Constructed wetlands have several advantages like high sustainability, low energy demand and much less attention and skill are needed for maintaining them. The design of these wetlands needs to be improved now and then to treat emerging pollutants like antibiotics, pharmaceutical products, and antibacterial resistant genome [154].

Various units work interactively in a constructed wetland for wastewater treatment. The components include a water holding basin, filtering substrate, growth medium for residing organisms, microorganisms, naturally developing aquatic invertebrates and most importantly, dense vegetation. Each component of a constructed wetland along with their respective functions is depicted in **Fig. 1.** The working efficiency of these wetlands depends on the integrated functioning of all of its components and their proper maintenance [155].

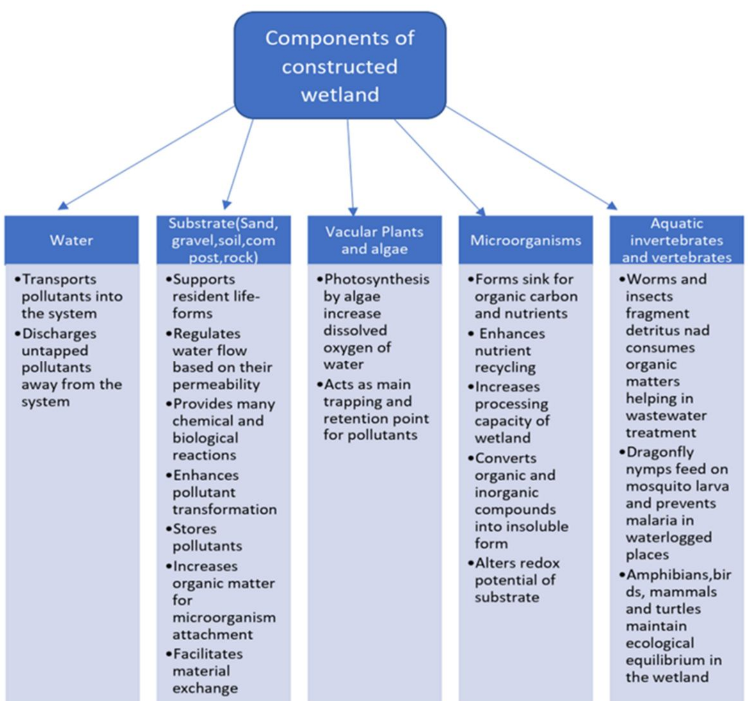

Fig 1: Components of constructed wetland with respective function of each

Fig 2 depicts water quality improvement through constructed wetlands involving several interrelated processes. These are - settlement of suspended particles, filtration, precipitation by exposing water to litter, adsorption, biotransformation, ion exchange on plant's and substrate's surfaces, microbial breakdown and modification of contaminants and their uptake by the plants, nutrient transformation by microorganisms and vegetation and finally the natural degradation of the pathogens [156].The mechanism of removal of contaminants within the constructed wetlands can occur individually, in sequence or simultaneously on each group of pollutants [157]. For example, Volatile Organic Compounds (VOC) present in the polluted groundwater are eliminated primarily by diffusion and volatilization. Other mechanisms like adsorption, photochemical oxidation, and biological reactions may also have a significant role. In a constructed wetland, physical reactions are responsible for the settling down, sedimentation and also volatilization of pollutants; whereas, gravitational settling down of pollutantants is responsible for elimination of solid suspensions [126].

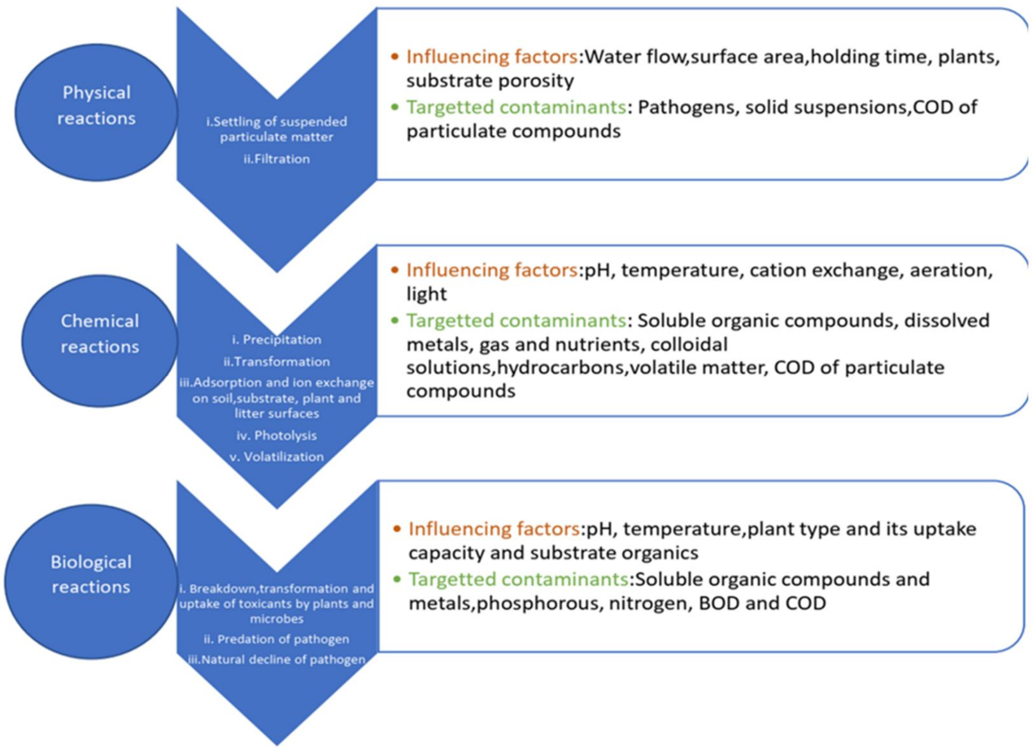

Fig 2: Steps of wastewater treatment in constructed wetlands

VI. CONCLUDING REMARKS

It is crucial to control the sources of pollution and perform regular environmental monitoring and assessment of the aquatic ecosystem keeping in mind the health of the endemic organisms specially. To achieve sustainable development goals, emphasizing the fourth phase of wastewater treatment is of utmost importance as traditional wastewater treatments cannot efficiently remove emerging micro-pollutants. So, it is a challenge for the researchers to gain detailed knowledge regarding different pollutants, including emerging ones, and to come up with strategies to remove their residues from water and ascertain the well-being of the environment in a wholesome manner. Bio-indication of aquatic ecosystem using fish will act as an effective environmental monitoring tool for pollution control and water body restoration and management.

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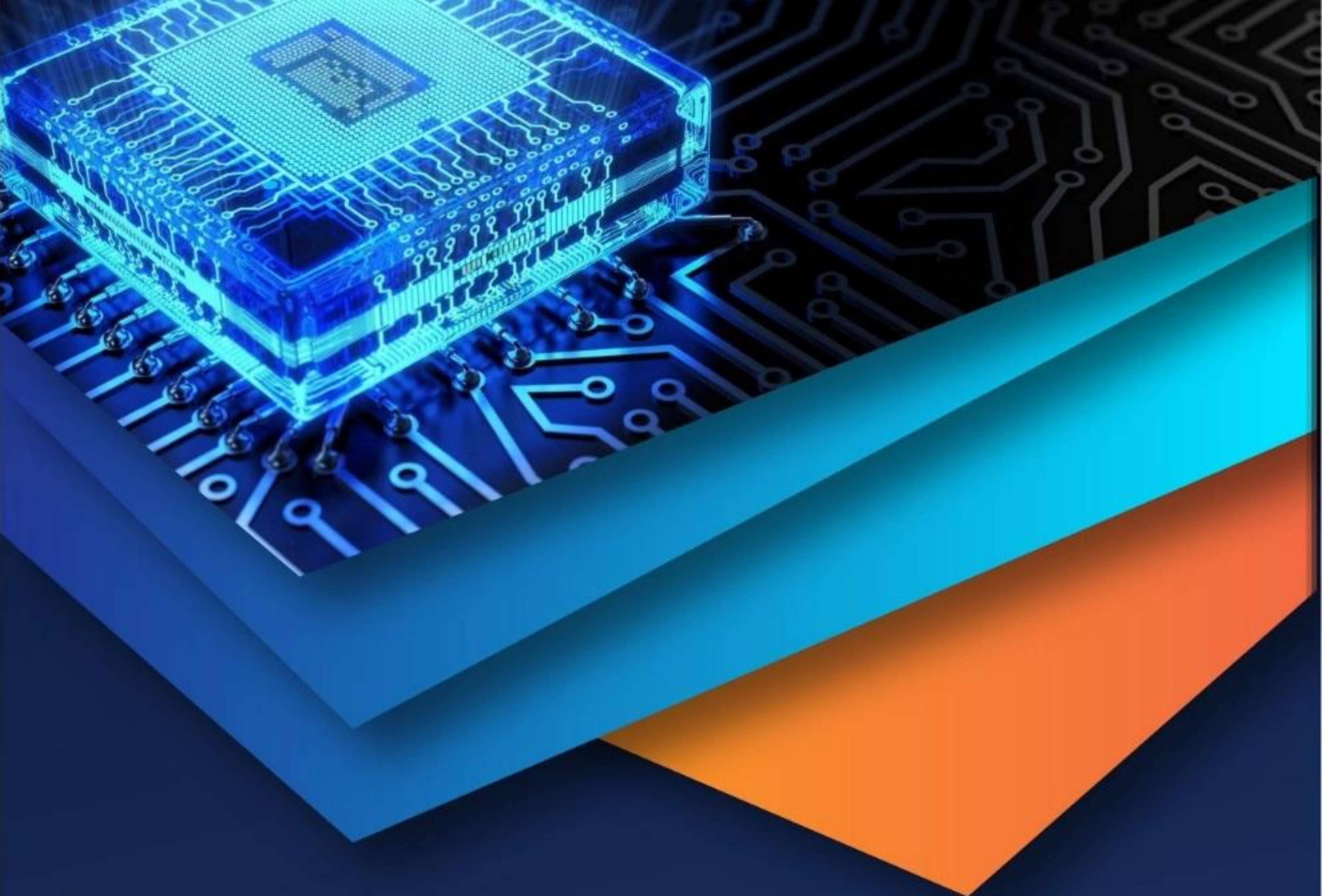

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