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Title: Relevance of genic expression studies to understand pollutants biodegradation

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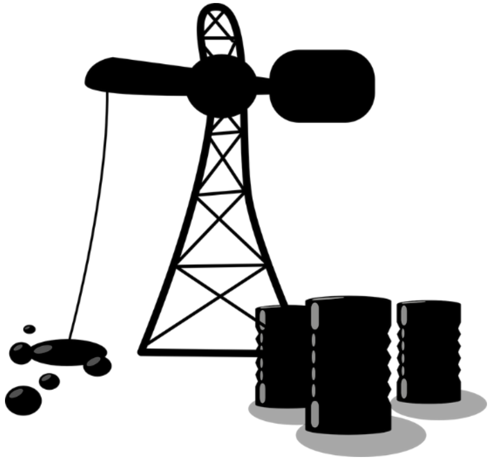
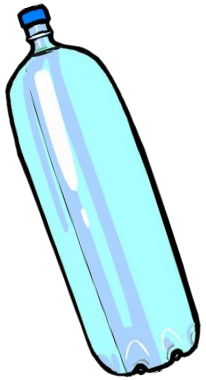
Introduction

Environmental pollution is defined as introducing the environment (air, water or soil) of substances harmful in higher than usual concentrations that reduce the quality (Manisalidis *et al*., 2020).



Hydrocarbons



Synthetic polymers

Heavy metals

Pesticides

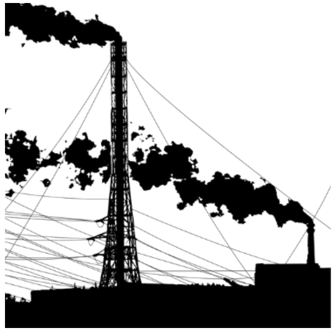
(Mishra *et al*., 2019)

Environmental impacts

Sources

Household

Agricultural

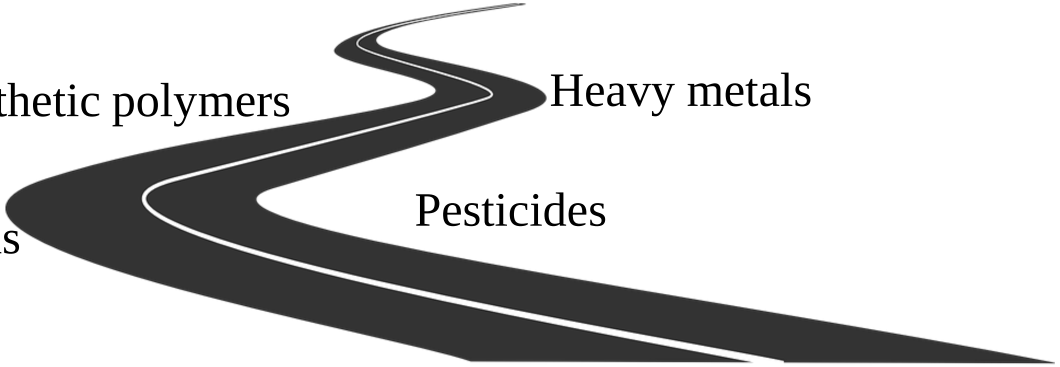


Industrial

Transportation

Synthetic polymers

Heavy metals



Hydrocarbons

Pesticides

Environmental and health human impacts

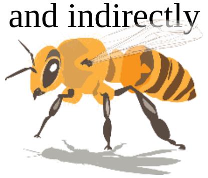
(Mishra *et al*., 2019)

Environmental impacts

Pollutant	Example	Source	Environment and human health impact	Reference
Hydrocarbons	Naphthalene Fluorene Phenanthrene Anthracene Xylene Toluene	Petrochemical industry Oil leaks Industrial activities as asphalt production Vehicle exhaust gases Forest fires	Toxic effects on flora and fauna Bioaccumulation along the food chain Carcinogenic, teratogenic and mutagenic They have been found in the human liver, kidney, lung, plasma and tissues Cardiovascular disorders	Marris *et al.*, 2020 Ahmed and Fakhruddin, 2018 Alegbeleye *et al.*, 2017
Synthetic polymers (plastics)	Polyethylene (PE) Polyethylene terephthalate (PET) Polyvinyl chloride (PVC) Polystyrene (PS) Microplastics (<5 mm)	Public waste Sewage, river and stormwater discharges Aquatic transportation Commercial and recreational fishing equipment	Fauna trapped or suffocated Ingestion and bioaccumulation in organisms Toxic to reefs Adverse effects on growth, photosynthesis, reproduction and immune system of organisms Release of toxic compounds 	Ganesh-Kumar *et al.*, 2019 Chae *et al.*, 2018 Rhodes, 2018

Environmental impacts

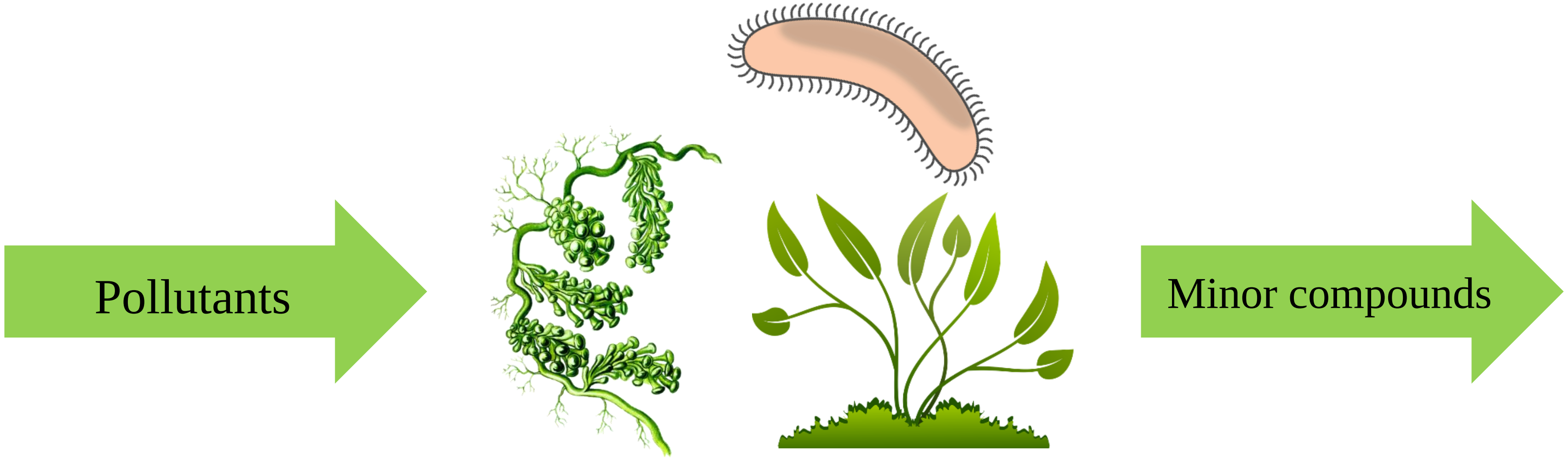
Pollutant	Example	Source	Environment and human health impact	Reference
Heavy metals	Mercury Copper Zinc Nickel Lead Cadmium Chromium Cobalt Arsenic	Metallurgical and glass industry Carbon-burning and other fuels Vehicle exhaust gases Municipal and industrial wastewater Paints	Damage to the membrane, proteins and DNA Interference with enzymatic activity Carcinogenic, neurotoxic and nephrotoxic Affect prenatal development and childhood Cause of cardiovascular disease, immune and reproductive disorders and alteration in blood composition	Zwolak *et al.*, 2019 Vareda *et al.*, 2019 Vardhan *et al.*, 2019
Pesticides	Carbamates Organophosphates Organochlorines Pyrethroids Triazines	Agriculture and irrigation Pest control activities Maintenance of private gardens Seeping Industrial wastewater Volatilization	Disruption depredator-prey They affect small fish directly and indirectly Interference with soil fertility Mutagenic, carcinogenic and neurodegenerative Cause of tumors, nervous system disorders, pulmonary dysfunction, immune system deficiency, cardiovascular, respiratory, kidney, endocrine, reproductive, and blood disorders	Hassaan *et al.*, 2020 Kaur *et al.*, 2019 Yadav and Devi, 2017



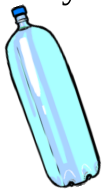
Biodegradation

Biodegradation is a biochemical process that refers to the broken down of various pollutants into more minor compounds caused by the metabolic potential of different organisms (Kour *et al.*, 2021; Alshehrei, 2017).

Bioremediation is the application of biodegradation to hydrolyze environmental contaminants (in soil, sediments, groundwater) to reduce levels below concentration limits established by regulatory authorities (Singh *et al*., 2014; Kensa, 2011).



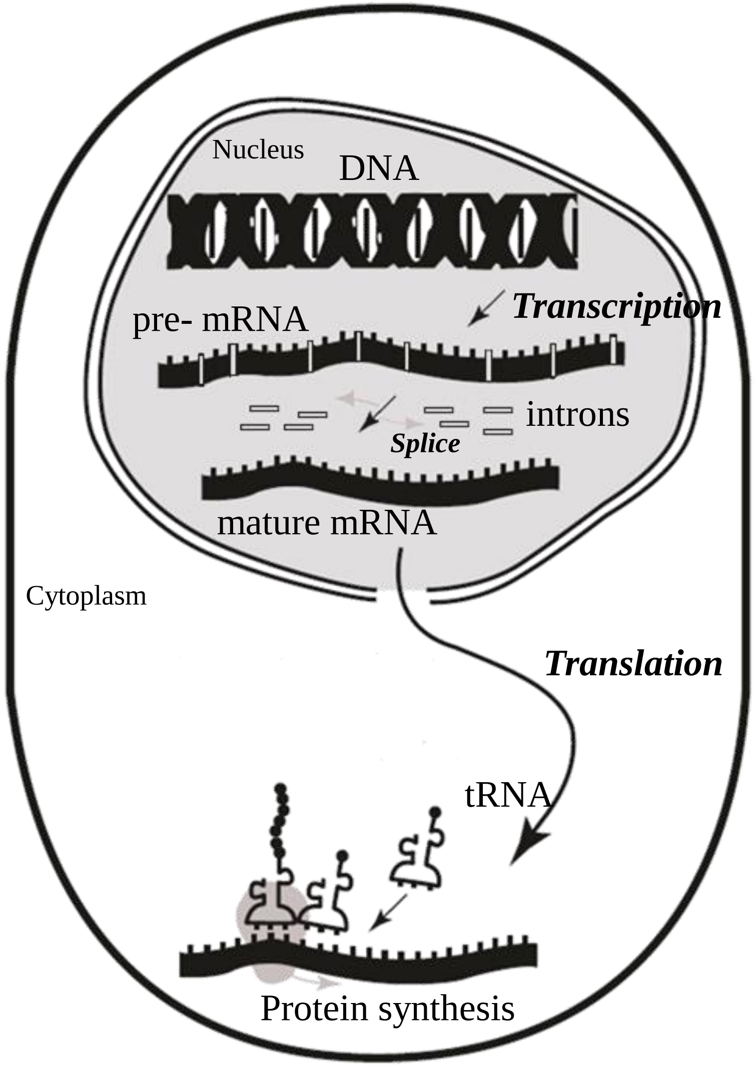
Biodegradation

Pollutant	Organism	Substrate	Reference	Mechanisms
Hydrocarbons 	*Acinetobacter* sp.	Total petroleum hydrocarbons	Cai *et al.*, 2021	The initial attack is generally through attachment to the substrates or production of biosurfactants/bioemulsifiers. The intracellular attack is an oxidative process (oxygenases and peroxidases), then peripheral degradation pathways convert HC into intermediates of central metabolism: β-oxidation and tricarboxylic acid cycle (Varjani, 2017).
	Pseudokirchneriella subcapitata	1-methylphenanthrene and 3,6-dimethylphenanthrene	Luo *et al.*, 2020	
	Klebsiella pneumoniae	Alkanes C_{10}-C_{20} in petroleum	Ozyurek and Bilkay, 2017	
	Pseudomonas aeruginosa	Total petroleum hydrocarbons	Varjani and Upasani, 2016	
	Bacillus sp.	Anthracene, naphthalene, benzene, toluene, xylene	Bisht *et al.*, 2014	
	Aspergillus terreus	Naphthalene and anthracene	Ali *et al.*, 2012	
Synthetic polymers (plastics) 	*Tenebrio molitor*	Polyvinyl chloride	Peng *et al.*, 2020	Microorganisms attack the polymer surface, and the extracellular enzymes secreted cause the main chain to cleave. The lower molecular weight compounds formed can be used by the microorganisms as carbon and energy source (Alshehrei, 2017).
	Streptomyces albogriseolus	Polyethylene	Shao *et al.*, 2019	
	Zophobas atratus	Polystyrene	Yang *et al.*, 2019	
	Brevibacillus sp. *Aneurinibacillus* sp.	Polypropylene	Skariyachan *et al.*, 2018	
	Aspergillus nidulans	Polyethylene terephthalate, polybutylene succinate, polycaprolactone, polylactic acid	Peña-Montes *et al.*, 2017	
	Ideonella sakaiensis	Polyethylene terephthalate	Yoshida *et al.*, 2016	

Biodegradation

Pollutant	Organism	Substrate	Reference	Mechanisms
Heavy metals 	*Bacillus cereus*	Cd, Cu, Ag, Zn	Al Azad *et al.*, 2020	Two mechanisms exist the neutralization of metals to non-toxic forms by the enzymatic attack (oxidoreductases, oxygenases, peroxidases) and bioaccumulation of heavy metals inside cellular components with non-apparent toxic effect (Ojuederie and Babalola, 2017).
	Saccharomyces cerevisiae	Pb, Cd, As, Hg	Massoud *et al.*, 2019	
	Mixed culture: *Desmodesmos* sp., *Chlorella* sp., *Scenedesmus* sp.	Al, Cu, Fe, Mn, Zn	Aslam *et al.*, 2019	
	Robinia pseudoacacia	Zn. Cd, Pb	Fan *et al.*, 2018	
	Gemella sp., *Micrococcus* sp. *Hafnia* sp.	Cd, Cr, Pb	Marzan *et al.*, 2017	
	Penicillium simplicissimum	Mo, V, Mn, W, Zn	Anahid *et al.*, 2011	
Pesticides 	*Chlamydomonas reinhardtii*	Trichlorfon (TCF)	Wan *et al.*, 2020	Degradation by extracellular enzymes is through oxidized, dehydrogenation, reduction or hydrolysis. Degradation by intracellular enzymes begins with the adsorption of pesticides on the surface of microbial cells. The membrane permeability determines its penetration of the cell and can be degraded by mineralization or partially degraded by co-metabolism (Ye *et al.*, 2018).
	Aspergillus flavus	Malathion	Derbalah *et al.*, 2020	
	Pseudomonas nitroreducens	Chlorpyrifos	Aswathi *et al.*, 2019	
	Pleurotus ostreatus	Aldrin, dieldrin	Purnomo *et al.*, 2017	
	Stenotrophomonas sp.	1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT)	Pan *et al.*, 2016	
	Trichoderma viride FRP3	Glyphosate	Arfarita *et al.*, 2016	

Gene expression



Reference: Selzer *et al.*, 2018

Genome
Genomic DNA

Transcription

Transcriptome
mRNA

Translation

Proteome
Protein

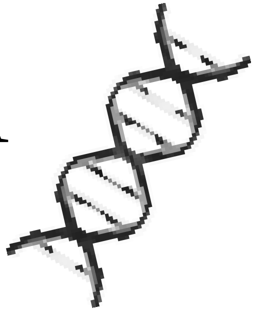
Regulation

- Chromatin remodeling
- Transcription
- Post-transcription
- Translation
- Post-translation

(Liang *et al.*, 2019; Spriggs *et al.*, 2010)

Omics sciences: a tool for gene expression study

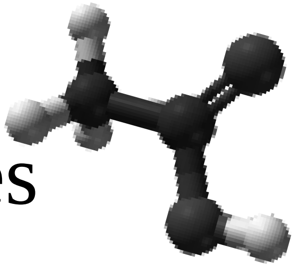
Genomic: DNA



Transcriptomic: RNA

Proteomic: Proteins

Metabolomic: Metabolites



Successful biodegradation research applying the study of gene expression

Pollutant	Organism used	Omic studies	Results	Contributions	Reference
Hydrocarbons (HC) Monocyclic aromatic hydrocarbon (MAH): aniline	*Delftia* sp. K82 isolated from the Gyeonggi province of Korea in 1992	Genomic: Next-generation sequencing (NGS) Transcriptomic: NGS Proteomic: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) For transcriptomic and proteomic, two cultures were prepared: Luria-Bertani media (LB) and aniline media (ANI)	Genomic: 6327 genes 6117 protein-coding genes Transcriptomic: 3919 genes were identified as differentially expressed genes Proteomic: ANI media 472 proteins LB media 409 proteins	Enzymes of the aniline degradation pathway and aniline-induced novel proteins were identified. ANI cultured was composed of 14 aniline degradation enzymes (11.9% of all proteins). Aniline oxygenase complex was induced more than 2-fold in aniline presence, transcriptionally and translationally. Among 95 proteins belonging to the cell wall, 12 were significantly induced in aniline media. Membrane proteins play essential roles in the protection against extracellular stress. *Delftia* sp. K82 has two different complete aniline degradation pathways.	Lee *et al.*, 2021

Successful biodegradation research applying the study of gene expression

Pollutant	Organism used	Omic studies	Results	Contributions	Reference
Synthetic polymer (plastic) Forms of polyethylene (PE): 1. PE4K: commercial powder 2. PE4K-OX: PE4K thermo-oxidized 3. PEfi: PET films 4. PEfi-OX: oxo-degradable film	*Rhodococcus ruber* C208 (environmental strain)	Transcriptomic: NGS Metabolomic: Lipidomic strategy by nano-electrospray ionization mass spectrometry (nano-ESI MS) Condition reference: Mannitol	Transcriptomic: 11 transcripts were commonly overexpressed in the presence of PE 39 transcripts could be directly assigned to alkane degradation and β-oxidation 34 transcripts encoding putative cytoplasmic oxidase A diacylglycerol kinase was detected Metabolomic: Three main lipid species could be observed	The most upregulated pathways in the presence of PE are alkane degradation and β-oxidation of fatty acids. Oxidases could well participate in the intracellular fragmentation of oxidized PE and extracellular oxidases to reduce the molecular mass of external PE. The redistribution of the phospholipid pattern and the presence of the enzyme diacylglycerol kinase suggests that PE fragments might serve as substrates for the β-oxidation pathway. Metabolic limiting steps were identified which could be fruitfully targeted for optimized PE consumption by *R. ruber.*	Gravouil *et al*., 2017

Successful biodegradation research applying the study of gene expression

Pollutant	Organism used	Omic studies	Results	Contributions	Reference
Heavy metals Plants were irrigated with 1000 mL of heavy metals contaminated water containing Cd, Pb, Cu and Ni at a concentration of 10 ppm. Reference plants were irrigated with tap water.	*Sorghum bicolor* (Sorghum) growing in an open glasshouse at Assiut University Experimental Farm, Assiut, Egypt	Transcriptomic: Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) in leaves of 12-weeks old plants irrigated either with tap-water or heavy metals contaminated water	Transcriptomic: The expression levels of all 15 genes were highly upregulated in response to heavy metals stress	SbZFP17, SbZFP346 and SbZFP6 genes are zinc finger proteins that are highly expressed in response to the stress imposed by heavy metals. SbLysMR1 plays a role in recognizing symbiotic bacteria, and it is induced in Cd, Cu, and Cr response. LAC9 (laccase gene) is expressed in response to the high concentration of Cu, Pb and Cd MAPKK gene plays a role in signal transduction of abiotic and biotic stress SbAVPL1 regulates solute transport across the vacuolar membrane of plant cells and plays a crucial role in accumulating heavy metals.	Abou-Elwafa *et al.*, 2019

Successful biodegradation research applying the study of gene expression

Pollutant	Organism used	Omic studies	Results	Contributions	Reference
Pesticide Hexaconazole 98% (triazole fungicide) dissolved in acetone at a concentration of 50 mg L^{-1}.	*Sphingobacterium multivorum* from sewage activated sludge and soil from a pesticide factory producing hexaconazole	Genomic: NGS Transcriptomic: RT-PCR and NGS Metabolomic: Ultra performance liquid chromatography quadrupole-time of flight mass spectrometry (UPLC/Q-TOF MS)	Transcriptomic: It was detected the presence of 864 differential genes Aldehyde dehydrogenase, monooxygenase, RND transporters and ABC transporters were upregulated Metabolomic: Three interesting metabolites were identified 2-(2,4-diclorophenyl-1-(1H-1,2,4-triazol-1-yl) hexane-2,5-diol; 2-(2,4-dichlorophenyl) hexane-1,2-diol and 1H-1,2,4-triazole	Differential genes are mainly related to metabolism; most of them are concerned with carbohydrate metabolism , energy metabolism and amino acid metabolism. There are also six genes about xenobiotic biodegradation and metabolites. The reactions of oxidation, hydroxylation and substitution were involved during the degradation of hexaconazole. RND transporter may involve the exportation of toxic metabolites to maintain homeostasis of strain. ABC transporter may provide the essential phosphoric acid and amino acid to survive under a high concentration of hexaconazole.	An *et al.*, 2020

Conclusions

The study of gene expression of degradative microorganisms using omics technologies provides relevant information that could be used to implement or improve the biodegradation process on a large scale.

These studies have offered crucial information about biodegradation that can be used to know or understand genes transcribed, enzymatic activities, novel enzymes, survival mechanisms, secondary metabolites, hidden biodegradation pathways, exact metabolic pathways, and modifications of existing pathways under conditions of stress caused by pollutants.

The knowledge about the degradative organisms will help design or improve remediation strategies by manipulating the pathways adding or deleting one or more genes, incorporating new metabolic pathways into organisms, or modifying enzyme specificity and affinity. Also, the enzymes could be used to produce other high-value-added metabolites such as precursors for biotechnological or pharmaceutical products.

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