Characterization of Polycyclic Aromatic Compounds, a Persistent Organic Pollutant, after Secondary Treatment of Pulp and Paper Sludge

Article title: Characterization of Persistent Organic Pollutants and Culturable and Non-Culturable Bacterial Communities in Pulp and Paper Sludge after Secondary Treatment
Authors: Sonam Tripathi, Sangeeta Yadav, Diane Purchase, Kaman Singh, Hind A. AL-Shwaiman, Ram Chandra
Reference: Sonam Tripathi, Sangeeta Yadav, Diane Purchase, Kaman Singh, Hind A. AL-Shwaiman, Ram Chandra, 2022. Characterization of persistent organic pollutants and culturable and non-culturable bacterial communities in pulp and paper sludge after secondary treatment, *Chemosphere*, *Volume 295, 133892*. ISSN 0045-6535.
https://doi.org/10.1016/j.chemosphere.2022.133892

Literature Assignment
Nicole Hanna
CHEM 3140
April 6th, 2023

Goal of the Research:

The goal of this research was to determine the persistent organic pollutants (POP's) present in pulp and paper sludge (PPS) after secondary treatment, obtained from a pulp mill in India, and determine their impact on microbial communities, specifically culturable and non-culturable bacteria, in PPS. This study aims to validate the efficiency of secondary treatment methods, help biodiversity research and ecological management for sustainability and environmental impacts. The identification of the POP's will provide information for proper disposal to prevent harmful risks to the environment associated with POP's.

Analytical question:

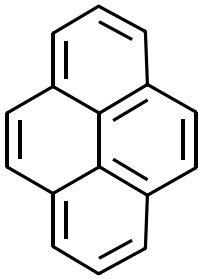
Can secondary treatment methods of pulp and paper sludge discharged from the Century Pulp Paper Mill in India aid in the identification of persistent organic pollutants present in the sludge by solvent extraction using gas chromatography-mass spectrophotometry?

Analytes and their General Properties:

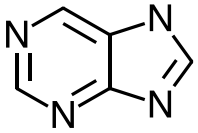
Polycyclic aromatic hydrocarbons

While multiple analytes were identified in this literature, polycyclic aromatic hydrocarbon compounds will be the focus of this assignment. Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds with two or more benzene rings in various structural configurations, containing only carbons and hydrogens. PAHs consist of two to *n*-rings, arranged in a planar aromatic structure that result from incomplete combustion of organic compounds. They are similar in structure to polychlorinated biphenyls (PCBs), minus the chlorination and are uncharged and non-polar. PAHs naturally occur in coal, crude oil and gasoline and can result from the burning of coal, oil, gas, wood, garbage, and tobacco; they are capable of binding and

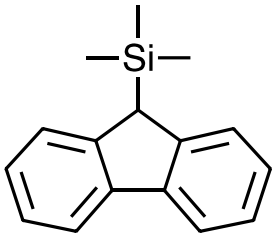
forming particles in the air. Light PAHs contain up to four rings, while PAHs with four or more rings are referred to as heavy; heavy PAHs are more stable and toxic than light PAHs. Based on this, PAHs would be produced during the processing of paper and pulp, due to wood burning and chemicals needed to process materials. PAHs can cause negative impacts on ecosystems and human health. In high exposure environments, liver and blood problems can occur. The three main polycyclic aromatic compounds analyzed in this research were purine, pyrene and 9-(trimethylsilyl). Purine is water soluble; pyrene is typically yellow; 9-(trimethylsilyl) is usually white (PubChem; ThermoFisher). Below are their general structures. Researchers state that PAHs require more attention due to their high prevalence in the atmosphere and negative impacts on human health and ecosystems. (CDC; Lawal, 2017).



Pyrene
$C_{16}H_{10}$
202.25 g/mol[a]



Purine
$C_5H_4N_4$
120.11 g/mol[b]



9-(trimethylsilyl)
$C_{16}H_{18}Si$
238.40 g/mol[c]

.

Analytical Method Steps:

Sample collection

Samples were randomly collected from the M/s Century Pulp Paper Mill in Uttarakhand, India. Random sampling would prevent researcher bias and allow for equal probability sampling of the object. Triplicate samples weighing 20 kg each were collected and stored in polyethylene bags in the lab at field humidity levels, until analysis occurred. Triplicate samples would provide validity of the research. Polyethylene bags were used as they can hold more samples than a bottle

could, and they are airtight storage vessel to prevent contamination. PPS contains inorganic substances, including calcite mud, and cellulose fibers which are both absorbent and can harden if not kept humid (Haile et al., 2021). Storage at field humidity levels would prevent the sludge from solidifying at low temperatures and keep the sludge moist until analysis time. A higher temperature may evaporate too much of the liquid in the sludge. Researchers did not state specific temperatures/parameters.

Sample preparation

A 50 g PPS sample was dried, sieved, and extracted with ethyl acetate using microwave assisted Soxhlet. The samples were diluted in 500 mL distilled water. This mixture was shaken at 100 rpm for 24 hours. The suspended beads were removed using a centrifuge at 3000 xg for 20 minutes. The supernatant pH was adjusted and maintained at 2.0, using 1 M hydrochloric acid (HCl). The organic contaminants were then extracted with ethyl acetate and DCM in equal volumes, by liquid-liquid extraction in a 500 mL separatory funnel (Yadav & Chandra, 2018). The upper layer of organic contaminants was kept, and dried under a vacuum at 40°C, which was further dissolved after drying in 1 mL acetonitrile suitable for the HPLC and filtered using a 0.22 μm syringe filter. All sampled were kept at 5°C until analysis.

Drying the samples was done to ensure proper analyte concentration was reported, as variable sample mass due to excess water can interfere. Sieving the samples would ensure the same particle sizes were being analyzed, which would also help break the sample into smaller particles for faster and easier dissolving. The particles can then be classified based on their particle size, and samples would be considered homogenous. Microwave assisted Soxhlet was used as it is allowing the liquid sample to cycle through multiple times, accelerated by microwave energy for better extraction. Ethyl acetate was found to be the best solvent choice for

PAH's and for identification on the GC-MS, based on a 2018 article published by Yadav & Chandra. The pH adjustment would ensure the extraction in later steps is optimal with the solvents, in this case, neutral PAHs were being extracted, therefore the pH would need to favour the dominant form of the acid. Liquid-liquid extraction would help the transfer of solutes from one phase to another, usually by solute partitioning; the organic phase can be easily kept. Drying the samples removes the excess liquid present, for suitable analysis in the HPLC with acetonitrile, while the filtration using a 0.22 μm filter would divide the dissolved and suspended samples. The low temperature of 5°C ensures no variability of samples due to excessive heating or cooling.

Analysis

Analysis was carried out on a gas chromatography-mass spectrophotometry (GC-MS) instrument. Before analysis, samples were diluted with 100 μl dioxane and 10 μl pyridine prior to silylation with 50 1 trimethyl silyl and trimethyl chlorosilane. Samples were heated to 60°C and shook intermittently for 15 minutes. A 1μl aliquot of the prepared samples was then loaded into the instrument for analysis. The detected pollutants were compared by their mass spectra using the NIST library of available compounds, and their retention time values.

The GC-MS was chosen as it has shown previously to be successful in the identification and quantification of pollutants, according to Yadav and Chandra, in 2015. The coupled technique would increase sensitivity and detection. Intermittent shaking of samples would assist in dissolving the solutions and ensure homogeneity of solutions. Silylation is a derivatization technique, which changes the analyte into a suitable form for detection in the GC, typically requiring the analyte to be made volatile. It was proven to be the best method in this case for the GC-MS (Yadav & Chandra, 2015).

Quality Control

No obvious quality control steps were mentioned. Triplicate samples were taken, which would ensure accurate data and better representative samples can be analyzed from the larger batch sample. Another step mentioned was comparing data to permissible limits set by the Central Pollution Control Board, which would validate the results being analyzed, and ensure reasonable values are being presented.

Method Critiques:

Question: can secondary treatment methods of pulp and paper sludge discharged from the Century Pulp Paper Mill in India aid in the identification of persistent organic pollutants present in the sludge by solvent extraction?

Answer: Yes! The method used by researchers allowed for characterization and determination of organic pollutants present in pulp and paper sludge and confirmed the validity of secondary treatment of sludge. Although, tertiary treatment is still recommended to ensure safe disposal. They were also able to determine resistance to certain persistent organic pollutants in culturable and non-culturable bacteria's, which provides information on hazards to human, plant, microbial and environmental health as persistent organic pollutants can reach these populations through pulp and paper sludge. Additional treatments may be required for safe disposal, and additional research is required to minimize effects from sludge POP's. Pulp and paper mills may be able to use this research to further process their effluents after processing and ensure that negative environmental impacts will not be sustained after processing. Additional critiques are as follows:

- Researchers stated they took random samples from the mill; however, did not explain how the random samples were collected. Through searching a different paper mentioned in a different section (Yadav & Chandra, 2018), a clearer explanation of random sampling was given. But, as the method was not referenced in the sample collection section, it is unclear if they used this sampling technique, or completely changed it. They did not state if sludge was collected near or downstream of the mill, or if grab/composite samples were taken. Researchers should have described and stated how they sampled, maybe including locations for clarity and to ensure accurate representative samples were described.
- Researchers did not state what equipment was used during collection. Stating this would allow for reproducibility, but to also know how much contamination could have arisen from the equipment.
- The field humidity level was not stated. This is challenging to interpret due to unknown climates of India. Additionally, the humidity would be different depending on the time of year samples were collected, which was also not stated. Also, humidity could be from the surrounding air, or from the treatment plant. After a quick search, humidity levels in India and paper mills are quite variable and would affect the storage and further research. It would have been beneficial, especially to those not familiar with Indian weather climates or paper mill humidity's to list the field humidity levels for reproducibility and accuracy.
- Clean up's, blanks and recovery standards were not mentioned during the method or discussion. Blanks would be beneficial to include as they allow for determination of random errors and the source of error to be identified. For this research, I would have

included a field, transport, equipment (although not mentioned what equipment was used, I would include one regardless), method and instrument blank. Recovery standards would be beneficial to include with the GC-MS to know how much analyte was recovered and compare to a certified reference material (also not mentioned). Some interferents were mentioned in section 3.2, but no proposal of clean-up was mentioned. A common interferent most likely present was sulphur and could be easily cleaned up with a reaction using copper or something similar. Also, this research was primarily focused on bacteria and mentioned fatty acids; lipids could very well be interfering and needing to be cleaned up by saponification with a base, for example.

- In their method researchers did not include any equipment they used to perform their experiments. For example, when the solutions were intermittently shaken, it did not state if this was done using an instrument or by hand. This would be helpful information to know for reproducibility of the method.
- This method also did not mention anything about green chemistry, but I would rate it as being decently green. Since they are testing from the environment, no chemicals were needed to generate a reaction to research, and minimal chemicals were in the analysis. With respect to energy efficiency, energy was required for 24 hours and an additional 20 minutes, which probably could have been cut down by increasing the rpm's and spinning for less time. Since they were investigating disposal of PPS, I would assume they also disposed of the chemicals and sludge correctly, therefore reducing waste. They also used off-line analysis and microwave assisted techniques, which are considered green. Overall, this method is quite green and followed the 12 principles of green chemistry.

- Information regarding different countries, as well as paper mills would have aided in this study to see if POPs are present in higher concentrations at certain mills. As my father works in the pulp and paper industry, he has attested those regulations in Canada, some states, and some European countries are quite strict, while other countries that are considered less environmentally friendly might have looser regulations. The Central Pollution Control Board has adjusted the regulations for the pulping industry in India, but 'allowed' pollution levels and requirements were not clearly stated.

References

Haile, A., Gelebo, G.G., Tesfaye, T. *et al.*, 2021. Pulp and paper mill wastes: utilizations and prospects for high value-added biomaterials. *Bioresource. Bioprocess.* ***8***, 35. https://doi.org/10.1186/s40643-021-00385-3

Lawal, A. T., 2017. Polycyclic aromatic hydrocarbons. A review. *Cogent Environmental Science*, *3*(1). https://doi.org/10.1080/23311843.2017.1339841

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 553570, 9-(Trimethylsilyl)fluorene. Retrieved April 2, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/9-_Trimethylsilyl_fluorene.

Polycyclic Aromatic Hydrocarbons (PAHs) Factsheet, *National Biomonitoring Program*, *CDC*. (2019, May 24). https://www.cdc.gov/biomonitoring/PAHs_FactSheet.html#:~:text=Polycyclic%20aromatic%20hydrocarbons%20(PAHs)%20are

Yadav, S., Chandra, R., 2018. Detection and assessment of the phytotoxicity of residual organic pollutants in sediment contaminated with pulp and paper mill effluent. *Environ Monit Assess* ***190***, *581*. https://doi.org/10.1007/s10661-018-6947-1

Yadav, S., Chandra, R., 2015. Syntrophic co-culture of Bacillus subtilis and Klebsiella pneumonia for degradation of kraft lignin discharged from rayon grade pulp industry, *Journal of Environmental Sciences*. *Volume 33*, Pages 229-238. ISSN 1001-0742. https://doi.org/10.1016/j.jes.2015.01.018.

Website of Central Pollution Control Board. *National Portal of India*. (n.d.). https://www.india.gov.in/official-website-central-pollution-control-board

9-(Trimethylsilyl)fluorene, 99%, *Thermo Scientific Chemicals*. (2023). https://www.thermofisher.com/order/catalog/product/B21816.06

Structures:

Generated on ChemDoodle using information from PubChem.

a: National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 31423, Pyrene. Retrieved April 5, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/Pyrene.

b: National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 1044, Purine. Retrieved April 5, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/Purine.

c: National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 553570, 9-(Trimethylsilyl)fluorene. Retrieved April 5, 2023, from https://pubchem.ncbi.nlm.nih.gov/compound/9-_Trimethylsilyl_fluorene.