

Hg and Pb Detection Utilizing d-Limonene from Sweet Oranges (*Citrus Sinensis*) Peelings

Stephen Kier S. Ponteras^a, Crijamaica L. Oceña^{a,b}, Chosel P. Lawagon^{a,b,*}

^a Chemical Engineering, College of Engineering Education, University of Mindanao, Davao City 8000, Philippines

^b Center of Green Nanotechnology Innovations for Environmental Solutions, Research and Publication Center, University of Mindanao, Davao City 8000, Philippines

clawagon@umindanao.edu.ph

Heavy metals contamination in bodies of water near mining sites is still prevalent. However, the challenge is monitoring contaminants in hard-to-reach areas, which can only be addressed using on-site and pretreatment-free detection of heavy metals. Herein, a simple Hg and Pb detection membrane was prepared using d-limonene and sulfur as by-products of orange zest and petroleum refining, respectively. The detection membrane included sulfur, which aids in the stabilization of limonene's ability to detect Hg and Pb. The coulometric system was used in the detection process. It was prepared by synthesizing limonene (from orange peelings) and integrating it in polysulfide. The as-prepared limonene polysulfide (LPS) was then coated into a membrane solid support. An LPS-polyvinyl alcohol (PVA) membrane was prepared via phase inversion technique. LPS typically have a dark red color, hence the coated PVA membrane adapted such color. The LPS-PVA membrane was exposed to an aqueous mercury solution, a bright yellow deposit formed on the polysulfide, which remains immobilized. Depending on the mercury concentration, the intensity of the color shift varied. A brighter color change was for Pb detection if the solution was alkaline. Hence, the results showed that waste orange peelings could be upcycled in an easy and simple Hg and Pb metals detection process, an intelligent waste management approach.

1. Introduction

The rapid development of urbanization and industrialization has led to pollution of terrestrial and aquatic environments which is regarded as a global issue (Irawanti et al, 2020). Mining, coal-fired power plants, and waste disposal are found to be the most significant industries that contributed to this pollution (Ali and Fatty, 2016). Though these developments have had a significant impact on local and national economies, large volumes of contaminants are released into the environment causing contamination in the downstream water, agricultural soils, food crops, and biota, as well as posing health risks to those living near those areas especially mining sites (Yan et al., 2020). Some of these contaminants occur naturally, however, they have increased dramatically as a result of anthropogenic sources, where heavy metal contamination is the major environmental concern among these effects (Srivastava et al., 2017).

Heavy metals are known to be non-degradable, highly persistent, toxic even at minute concentrations, and can cause adverse effects to the environment and human health (Sazon and Migo, 2020). Mercury, lead, and arsenic poisoning are some of the acute or chronic illnesses that may be developed when exposed to heavy metals (Gigantone et al, 2016). In a recent study, mercury (Hg) contamination was identified in at least four (4) areas in the Philippines including bodies of water and mining sites (Samaniago et al, 2020). Some of these areas are already abandoned but owing to the current mercury levels, they must be monitored. Lead (Pb) was also given focus as it reduces intelligence in children causing mental retardation and other health impacts over time (EcoWaste Coalition, 2017). These two heavy metals are widely prevalent in the Philippines although there are efforts for homeostasis mechanisms for them, regular detection and monitoring are essential to ensure the safety of the people especially in water sources near mining areas (Gutierrez, 2016). However, bulky and expensive analytical techniques are used to identify trace-level harmful heavy metal ions like Hg and Pb including high-performance liquid chromatography (HPLC) coupled with electrochemical- or UV-Vis-detectors,

wet chemical methods, etc (Shimizu et al, 2019). Hence, there is a need for portable, precise, quick, sensitive, and cost-effective detection methods where these can be utilized in communities and regions near small-scale mining areas. A simple approach of utilizing two industrial by-products, sulfur from the petroleum refining industry and d-limonene oil from orange zest in the citrus industry, are explored to produce limonene polysulfide (LPS) in an attempt to provide this cost-effective detection kit (Crockett et al, 2016). These by-products are inexpensive and results are promising and could be used in heavy metal detection especially Hg and Pb. However, LPS should have a solid support to render it processable for efficient and easy detection of heavy metals contaminants in wastewater.

Herein, polyvinyl alcohol (PVA) was used as primary solid support by integrating it with LPS via phase inversion technique. This LPS-PVA membrane material can be an innovative way to address the challenges in detecting Hg and Pb using bulky and expensive analytical techniques. Moreover, the outcome of this study will help people living near mining areas and suspected heavy metal contamination of bodies of water to rapidly detect these heavy metals.

2. Materials and Methods

2.1 Materials

Orange peelings were procured from a local wet market in Davao City, Philippines. Sulfur ($\geq 99.0\%$, Sigma-Aldrich, USA) and as-prepared limonene oil were used for the LPS synthesis. Polyvinyl alcohol (99+%, 85,000-124,000MW, Sigma-Aldrich, USA) and acetone (99.5%, 58.08MW, Ajax Finechem Pty Ltd, Australia) were utilized for LPS membrane preparation. Mercury (II) chloride (99.5%, 271.50MW, Scharlau Chemie S.A, Spain), Lead (II) chloride (98%, 278.1MW, Asia Pacific Specialty Chemicals Ltd., Australia), zinc sulfate heptahydrate (99.5%, 287.54MW, HiMedia Laboratories Pvt. Ltd., India), copper (II) sulfate pentahydrate (99-100.5%, 249.68MW, Scharlab S.L., Spain), and cadmium sulfate (208.48MW, Mallinckrodt Baker Inc., USA), were used for the preparation of simulated wastewater.

2.2 Characterization

The concentration of limonene was determined via UV-Vis (BK-D560 Spectrophotometer, China) utilizing methanol as the spectroscopy blank and 228 nm wavelength. Surface morphologies of the LPS and composite LPS membrane samples were observed under Scanning Electron Microscope (ThermoScientific Quanta 250FEG SEM-EDX, USA). The SEM was equipped with an Energy Dispersive X-ray Spectrometer (EDX) for elemental (C, S) mapping of the LPS membrane. Fourier Transform Infrared Spectroscopy (Bruker Tensor 2, USA) was used to identify the chemical bonds in the prepared samples.

2.3 Limonene oil extraction from waste orange peelings

Orange peelings (19.00 ± 2.41 g) were grated, separating the fibrous part, and mixed with deionized (DI) water (100 mL) in a round bottom flask. The mixture was heated in an oil bath at a constant temperature (up to 98 °C). About one drop of distillate each second can be observed, and an oil layer (~1.5 mL) was visible in the collected product.

2.4 Synthesis of limonene polysulfide (LPS)

Sulfur was melted ($T > 120$ °C) and an equal amount of limonene was added to molten sulfur then heated to 170 °C as a starting point. A two-phase mixture is formed producing a single dark red phase. The reaction was done for 5 minutes.

2.5 Preparation of LPS membrane

Phase inversion technique was used for the LPS membrane preparation. A PVA dope solution (9 wt. %) was stirred at 90 °C for 2 h and 30 °C for 10 h. As synthesized LPS was mixed (2 LPS:1 PVA by mass) into the solution and stirred for 15 minutes (60 °C). The polymer solution was then cast to a glass plate and kept there for 10 minutes before immersion in an acetone bath (10 minutes). The prepared LPS membrane was then air-dried for 12 h.

2.6 Color analysis

A Gaussian Blur was applied before the droplets were separated from the background and attached to a mask. The created mask was then evaluated for the amount of yellow pixel percentage detected. This is done using the Hue Saturation Value (HSV) scale with colors ranging from dark to light yellow [(8,40,10)-(35,255,255)]. All of these were designed and coded using the Jupyter Notebook through Anaconda with Python as the primary programming language. Included with the code were the libraries for OpenCV, Matplotlib, and Numpy.

3. Results and discussion

3.1 Characterization

Limonene ($99.98\% \pm 0.15$) was extracted from waste orange peelings with a yield of ~ 6.7 wt. % relative to the initial mass of orange peelings. It was then used to prepare LPS which was confirmed from the FTIR analysis (Figure 1a) to contain the valence vibration of the C-H bond and the characteristic peak of C=C, which all correspond to limonene (Derdar et al., 2019). It also has the distinct peaks of polysulfide: absorption bands of C-S, S-CH₂, and S-H from thiol groups (Mortazavian et al., 2019). The high carbon (C) and sulfur (S) content were verified by the EDX result (Figure 1b), indicating limonene and polysulfide, respectively. The SEM image (Figure 1c) showed that the LPS has film-like morphology which is expected since it is a viscous liquid. It also has uniform dispersion of the limonene and polysulfide (Figure 1d). On the other hand, the LPS-PVA membrane has a rough and fibrous structure (Figure 2a), and its EDX analysis (Figure 2b) showed high S content indicating the presence of LPS. The LPS was also uniformly dispersed in the PVA membrane, as shown by the C and S elemental map (Figure 2c, 2d). Hence, the phase inversion method was established as a facile method in utilizing PVA as solid support of the LPS.

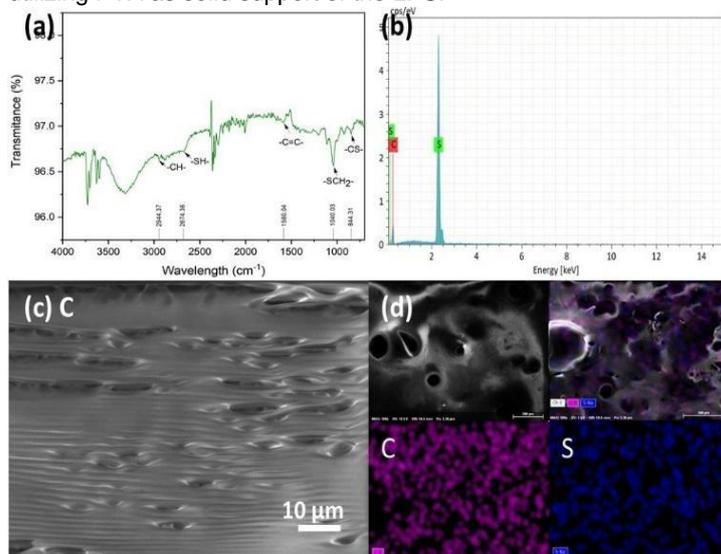


Figure 1: (a) FTIR, (b) EDX, (c) SEM micrograph, and (d) elemental mapping of limonene polysulfide.

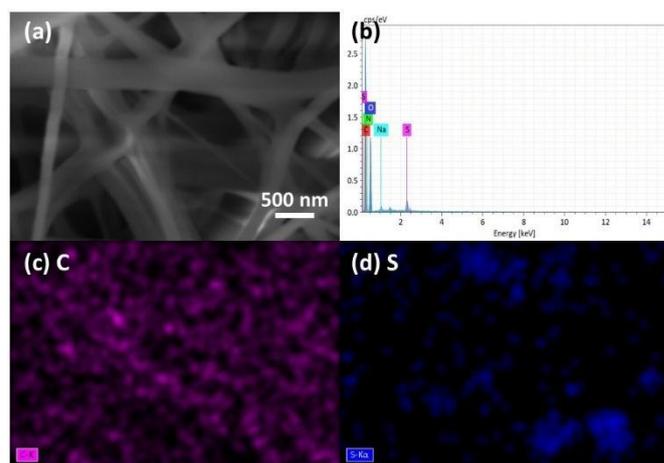


Figure 2: (a) SEM micrograph, (b) EDX analysis, and (c) C, (d) S elemental mapping of limonene polysulfide-PVA membrane.

3.2 Effect on simulated heavy metal wastewater

Exposure to heavy metals, especially Hg^{2+} and Pb^{2+} , is a health hazard (Sazon and Migo, 2020) often found in wastewater or bodies of water near mining sites. Hence, inexpensive technologies to determine Hg^{2+} and Pb^{2+} contamination will be helpful. LPS can be used for selective heavy metal detection. As observed (Figure 3), no significant color change was observed when the LPS was dropped separately with aqueous solutions containing Cd^{2+} , Cu^{2+} , Zn^{2+} . But when a simulated heavy metal contaminated wastewater (containing Hg^{2+} , Pb^{2+} , Cd^{2+} , Cu^{2+} , and Zn^{2+}) was used (Ling et al., 2011) a bright yellow reaction was observed. The chromogenic reaction started at 60 seconds, and the reaction became more intense as time passed, and not much significant change after 600 seconds until 1800 seconds. The color analysis also showed the same trend. Although, for Zn^{2+} , a very light color change was observed but not that prominent. Hence, selective chromogenic detection is possible for an aqueous solution containing Hg^{2+} , Pb^{2+} .

Time (sec)	0	10	20	30	40	60	120	300	600	1200	1800
LPS (Mix)											
Color analysis	0.00	4.13	5.84	11.59	32.17	40.09	41.18	54.73	65.42	66.01	66.01
LPS (Cd^{2+})											
Color analysis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LPS (Cu^{2+})											
Color analysis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LPS (Zn^{2+})											
Color analysis	0.00	0.00	0.00	0.00	0.00	0.00	0.11	3.86	5.47	6.24	6.56

Figure 3: Chromogenic reaction of other metals ($200 \text{ mg L}^{-1} \text{ Cd}^{2+}$, Cu^{2+} , Zn^{2+}) and simulated heavy metal wastewater (Mix) to limonene polysulfide (LPS) with respect to time.

3.3 Effect of heavy metal concentration

Concentration (mg L^{-1})	0.5	1	3	5	7	10
Hg^{2+}						
Color analysis	34.23	44.84	48.43	48.75	55.18	66.92
Pb^{2+} (pH = 7)						
Color analysis	0.00	0.00	0.00	0.00	0.00	0.00
Pb^{2+} (pH > 7)						
Color analysis	30.42	33.45	62.73	89.31	96.46	98.98
Pb^{2+} (pH < 7)						
Color analysis	0.00	0.00	0.00	0.00	0.00	0.00
Deionizer H_2O						
Color analysis	0.00					

Figure 4: Chromogenic reaction of varied concentration Hg^{2+} and Pb^{2+} to limonene polysulfide (LPS) after 30 minutes.

The varied concentration of Hg^{2+} and Pb^{2+} ($\text{pH} > 7$) aqueous solution has chromogenic reactions when added to the surface of LPS (Figure 4). The images were taken after 30 minutes of dropping the aqueous solutions to the surface of LPS. The color changed from transparent to light yellow, then darker yellow, as evidenced by the increasing yellow pixel percentage when the heavy metal concentration increases. However, the Pb^{2+} chromogenic reaction to LPS happens only when $\text{pH} > 7$ ($\text{pH} = 11$) with a brighter yellow deposit and higher yellow pixel percentage compared to that of the Hg^{2+} solutions. When the $\text{pH} < 7$, there is a color change but not as prominent when $\text{pH} > 7$. Hence, the presence of metal ions is influenced by pH value. Acidic solution ($\text{pH} < 7$) causes ionization of metal ions which strengthens the chromogenic reaction. Alkaline solution ($\text{pH} > 7$) precipitates the ions as observed by the bright yellow deposit but still maintains the chromogenic reaction (Na Kim et al., 2012). As a baseline value, when deionized water was dropped into the LPS surface, no reaction occurred, and it stayed transparent. Therefore, it can be surmised that Hg^{2+} and Pb^{2+} ions have a significant chromogenic reaction towards LPS at varied concentrations even as low as 0.5 mg L^{-1} or even possibly lower.

3.4 Effect of LPS PVA membrane

It was established that LPS has a chromogenic reaction to Hg^{2+} and Pb^{2+} . Although for Pb^{2+} , the pH value must be changed for the color change to occur. Nevertheless, solid support for LPS will enable easy handling of the LPS for its utilization as a field detection kit for Hg^{2+} and Pb^{2+} . PVA was chosen as the primary membrane for solid support due to its hydrophilicity (Liu et al, 2019). The phase inversion method (Figure 5) was chosen to ensure a facile preparation and uniform dispersion of LPS in the membrane. This method can be completed in less than an hour. PVA is dissolved in DI water (polymer solution), cast on a glass plate, and immersed in a coagulation bath (acetone). DI water and acetone solvents exchanged, allowing precipitation to occur and producing the LPS-PVA membrane upon thoroughly drying.

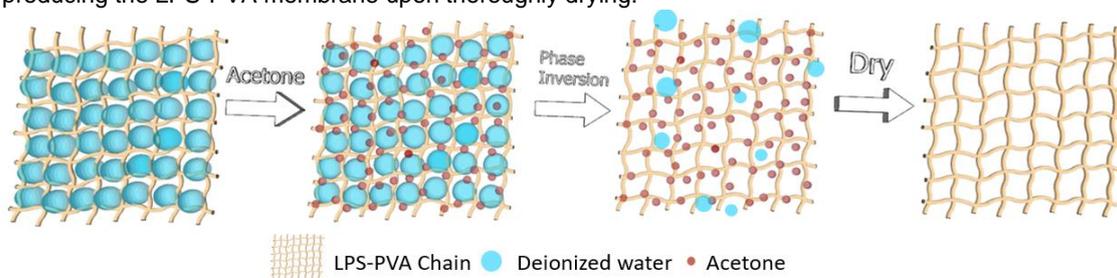


Figure 5: Schematic diagram of the phase inversion techniques used to prepare the LPS

Time (sec)	0	10	20	30	40	60	120	300	600	1200	1800
LPS (Hg^{2+})											
Color Analysis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.98	23.58	39.06	40.16
PVA-LPS (Hg^{2+})											
Color Analysis	0.00	0.00	0.00	76.03	85.58	89.39	90.24	95.04	97.76	97.83	98.64
LPS (Pb^{2+})											
Color Analysis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.74	31.53	39.73	57.49
PVA-LPS (Pb^{2+})											
Color Analysis	78.55	58.96	46.91	36.74	92.85	90.1	92.78	93.67	98.00	98.29	85.43

Figure 6: Chromogenic reaction of Hg^{2+} and Pb^{2+} (1 mg L^{-1}) to limonene polysulfide (LPS) and PVA-LPS with respect to time.

Testing the LPS-PVA membrane by dropping aqueous solutions of Hg^{2+} and Pb^{2+} resulted in a similar chromogenic reaction (Figure 6) to that of the LPS samples. Based on the color analysis, the yellow pixel percentage increased with respect to time. But notably, the yellow pixel percentage in the LPS-PVA membrane

started to appear earlier as compared to that of the LPS. It indicates that the Hg^{2+} and Pb^{2+} have a better affinity to LPS-PVA membrane than the LPS alone, which can be due to PVA's hydrophilicity. This result further indicates the suitability of PVA as the primary solid support of LPS while highlighting the efficacy of LPS's chromogenic reaction to Hg^{2+} and Pb^{2+} .

4. Conclusions

An LPS-PVA membrane was prepared for the selective chromogenic detection of Hg^{2+} and Pb^{2+} in aqueous solutions. A bright yellow deposit was formed once a solution containing Hg^{2+} was dropped, and the yellow pixel percentage increased as the concentration increased. For the Pb^{2+} , the pH value of the solution must be changed, and a higher yellow pixel percentage is observed for an alkaline solution. Hence, the LPS-PVA membrane can be used as a portable chromogenic Hg^{2+} and Pb^{2+} detection kit. It is an application worth delving into in future studies as the LPS-PVA is also very processable and can be used as a coating in wastewater treatment devices.

Acknowledgments

This study was supported by the Young Innovators Program (YIP) of the Department of Science and Technology - Philippine Council for Industry, Energy, and Emerging Technology Research and Development (DOST-PCIEERD).

References

- Ali M. M., Fatti L. P., 2006, A differential free point generation scheme in the differential evolution algorithm, *Journal of Global Optimization*, 35, 551–572.
- Crockett M. P., Evans A. M., Worthington M. J. H., Albuquerque I. S., Slattery A. D., Gibson C. T., Chalker J. M., 2016, Sulfur-Limonene Polysulfide: A Material Synthesized Entirely from Industrial By-Products and Its Use in Removing Toxic Metals from Water and Soil, *Angewandte Chemie - International Edition*, 55(5), 1714–1718.
- Derdar H., Belbachir M., Harrane A., 2019, A Green Synthesis of Polylimonene Using Maghnite-H+, an Exchanged Montmorillonite Clay, as Eco-Catalyst, *Bulletin of Chemical Reaction Engineering and Catalysis*, 14 (1), 69-78.
- EcoWaste Coalition, 2017, Lead in Solvent-based Paints for Home Use in the Philippines, IPEN, 1-50.
- Gigantone C. B., Sobremisana M. J., Trinidad L. C., Migo V. P., 2020, Impact of Abandoned Mining Facility Wastes on the Aquatic Ecosystem of the Mogpog River, Marinduque, Philippines, *Journal of Health and Pollution*, 10(26), 1–12.
- Gutierrez R., 2016, Philippine National Action Plan on the Phaseout of Mercury in ASGM, Artisanal and Small-Scale Gold Mining. United Nations Environment Programme, 1-21.
- Irawanti R., Koesmawati T.A., Lugitoa G., Zunitaa M., Graphene Oxide (Go) Membrane In Removing Heavy Metals From Wastewater: A Review, *Chemical Engineering Transactions*, 82, 415-420.
- Liu Y., Hu H., Yang X., Lv J., Zhou L., Luo Z., 2019, Hydrophilic modification on polyvinyl alcohol membrane by hyaluronic acid, *Biomedical Materials (Bristol)*, 14(5).
- Mortazavian S., Saber A., Hong J., Bae J-H., Chun D., Wong N., Gerrity D., Batista J., Kim K., Moon J., 2019, Synthesis, characterization, and kinetic study of activated carbon modified by polysulfide rubber coating for aqueous hexavalent chromium removal, *Journal of Industrial and Engineering Chemistry*, 69 (25), 196-210.
- Na Kim H., Xiu Ren W., Seung Kim J., Yoon J., 2012, Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions, *Chemical Society Reviews*, 41(8), 3210–3244.
- Samaniego J., Gibaga C. R., Tanciongco A., Rastrullo R., 2020, Total mercury in soils and sediments in the vicinity of abandoned mercury mine area in Puerto Princesa City, Philippines, *Applied Sciences (Switzerland)*, 10(13), 0–9.
- Sazon R. R., Migo V. P., 2020, Metal concentrations in sediments of the Alinsaog River, Santa Cruz, Zambales, Central Luzon, Philippines, *Journal of Health and Pollution*, 10(27).
- Shimizu F. M., Braunger M. L., Riul, A., 2019, Heavy metal/toxins detection using electronic tongues, *Chemosensors*, 7(3), 1–19.
- Srivastava V., Sarkar A., Singh S., Singh P., de Araujo A. S. F., Singh, R. P., 2017, Agroecological responses of heavy metal pollution with special emphasis on soil health and plant performances, *Frontiers in Environmental Science*, 5, 1–19.
- Yan C., Wang F., Liu H., Pu S., Lin F., Yuan R., 2020, Deciphering the toxic effects of metals in gold mining area: Microbial community tolerance mechanism and change of antibiotic resistance genes, *Environmental Research*, 189.