

INVESTIGATING PHOTOCHEMICAL EFFECTS OF MICRO-XRF ANALYSIS ON COMMON GEOCHEMICAL COMPOUNDS

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ABSTRACT

Chemical alteration of organic compounds exposed to X-rays from portable micro-focus X-ray fluorescence instruments is a potential concern for many applications (e.g. the analysis of valuable works of art, planetary lander and rover missions focused on astrobiology, etc.). We performed a preliminary study of the effects of intense and prolonged exposure to X-ray radiation from a micro-focus XRF instrument on the chemical composition of two organic compounds representative of molecules commonly reported from the terrestrial rock record. Pyrene, a polycyclic aromatic hydrocarbon (PAH), and palmitic acid, a saturated fatty acid, were deposited in glass vials and irradiated for 90 and 24 hours, respectively. Radiation was delivered using a Bruker ARTAX instrument fitted with a polycapillary focusing optic and Rh anode X-ray tube operated at 50 kV/0.6 mA. Controls and samples were compared for chemical alteration by Gas Chromatography/Mass Spectrometry (GC-MS) and Raman spectroscopy, before and after exposure to the X-ray beam. No indication of chemical alteration of the compounds was detected by GC-MS or Raman spectroscopy. A color change that was observed in the vials containing the samples after X-ray irradiation was likely caused by reversible activation of color centers in the borosilicate glass vials.

INTRODUCTION

Due to the importance of photo degradation of samples in the fields of medicine and chemistry, numerous publications have explored photochemical reactions occurring in biological samples (e.g. George *et al.*, 2012; Kempner, 2011). However, little is known about the effects of X-rays generated by micro-XRF instruments on organic molecules commonly preserved in rocks and soils. For the photon energy range relevant to this work (10 – 50 keV), there are two types of radiation damage that are of interest. Primary damage is caused by the direct interaction of incident photons with target electrons (e.g. Compton scattering and the photoelectric effect). Chemical bonds can break due to exposure to electrons energized and liberated by these processes. Also, secondary damage results from the formation and reaction of radiolytic products, such as free radicals, which have the potential to break chemical bonds (Teng and Moffat, 2000).

Micro-XRF instruments are becoming increasingly common tools used by field geologists. A mapping micro-XRF instrument for lithochemistry measurements has also been selected to fly on

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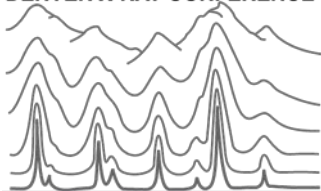
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an upcoming NASA's Mars rover mission (PIXL on Mars 2020; Allwood *et al.*, 2015), where it will make measurements in coordination with a UV Raman/fluorescence spectrometer (SHERLOC) that is capable of mapping the distribution of organics (Beegle *et al.*, 2015). Given the potential for these micro-XRF instruments to alter organics in situ, there is a need to investigate the photochemical effects of micro-XRF analyses on geological samples. In order to develop experimental procedures for this type of evaluation and make recommendations for future work, we conducted a preliminary investigation of the effect highly-focused X-rays at the upper limit of energies typically used by these instruments had on two common geochemical compounds: pyrene and palmitic acid. Controls and samples were compared for chemical alteration by Gas Chromatography/Mass Spectrometry (GC-MS) and Raman spectroscopy, before and after exposure to the X-ray beam. The organic compounds were prepared and irradiated through glass vials because keeping the vials sealed minimized contamination visible in highly-sensitive GC-MS analyses.

EXPERIMENTAL

We used a Bruker ARTAX rastering micro-XRF instrument fitted with a Rh anode X-ray tube. The ARTAX instrument uses a micro-focus X-ray tube and polycapillary optic to focus the X-ray beam down to a $<100\ \mu\text{m}$ spot. The instrument was operated at 50 kV and 0.6 mA, rather than the 28 kV used by similar instruments designed for planetary missions, in order to maximize the intensity of the X-ray beam that would be transmitted through the glass vial to increasing our chances of inducing photochemical alteration in the organic compounds.

Borosilicate glass vials with an inner diameter of 10 mm were used in this experiment to hold the samples. The vials were kept sealed during the irradiation using a screw thread cap. All chemicals were purchased from Sigma-Aldrich, Inc. The palmitic acid sample (Figure 1a) was prepared by dissolving 5 mg of the sample in 0.5 mL of hexane. The pyrene sample (Figure 1b) was immobilized by placing 0.5 mg of pyrene at the bottom of a vial which contained one drop of dichloromethane solvent. Both samples were left to dry prior to irradiation. Figure 1 displays the image of two samples captured using a 10x objective lens after irradiation.

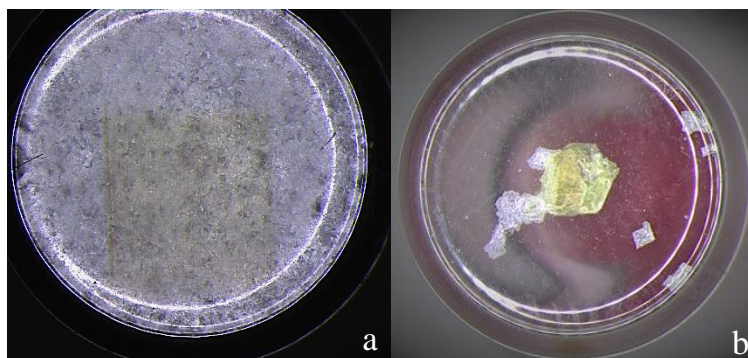


Figure 1a. Palmitic acid deposited in the glass vial. **Figure 1b.** Pyrene deposited in the glass vial. The yellow brown color seen in Figure 1a and Figure 1b are due to activation of color centers in the glass as a result of the displacement of lattice atoms or the production of electron hole pairs after X-ray irradiation (reddish color is the reflection of light from the stage of the microscope).

Millimeter-scale areas of the bottom of the vials were irradiated in a perpendicular position with respect to the laser beam (in air) for 24 hours (palmitic acid) and 90 hours (pyrene) through ~0.81 mm glass vials. Although a substantial percentage of incoming X-ray beam flux at lower-energies was absorbed by the glass, keeping the vials sealed minimized the potential for contamination that was detectable by our highly-sensitive GC-MS analyses. X-ray beam transmission increases drastically at higher energies. At our upper energy limit (50 keV), 94% of the beam flux passes through the 0.081 cm thick glass vial.

Irradiated areas for palmitic acid and pyrene were 25 and 2 mm², respectively. The instrument was rastered in 100 μm increments (step size) in order to irradiate as much of the sample surface as possible. The exposure time per spot was 27 seconds for palmitic acid, and 27 minutes for pyrene. Aromatic compounds, such as pyrene, are usually more resistant to ionizing radiation when compared to aliphatic compounds. This is due the resonance properties of a stable carbon ring system (Egerton *et al.*, 2004). As such, we subjected pyrene to an irradiation time exceeding that of palmitic acid.

After exposure to the X-ray beam, samples were analyzed by GC-MS and Raman spectrometry for indications of potential chemical alteration. Raman analyses were performed first, through the bottom of the sealed glass vials, using a dispersive Raman spectrometer (Horiba Jobin-Yvon LabRam HR) configured for 0.4 cm⁻¹ resolution using an 1800 grooves/mm grating. Samples were excited by a 532-nm Nd:YAG laser operating at 50 mW output. A silicon chip was used for frequency calibration and was found to exhibit a well-defined sharp peak at 520.7 cm⁻¹.

Samples were then opened and analyzed by GC-MS using a Trace Ultra GC (Thermo Scientific) connected to an ISQ Series quadrupole mass spectrometer (Thermo Scientific). Helium was used as the carrier gas, and the GC was fitted with a 30m DB-5 column (Agilent). For palmitic acid, the irradiated sample was dissolved in 3 ml of hexane in the vial. After removing 2.8 ml of the solution from the vial, the remaining 0.2 ml solution was diluted to 3 ml by adding hexane. For pyrene, the 0.5 mg of the sample after irradiation was dissolved in 3 ml hexane / dichloromethane mixture in the vial. After removing 2.5 ml of the solution, the remaining 0.5 ml was diluted to 3 ml by adding solvent. One microliter of each sample in hexane was injected in splitless mode onto the column which was held at 60°C for two minutes, ramped to 150°C at 10°C per minute, then to 315°C at 3°C per minute where it remained for 20 minutes. We note that due to the shape of the vials, the available mapping modes of the micro-XRF instrument, and our desire to limit contamination, we were unable to avoid non-irradiated parts of the sample being mixed into the post-irradiation GC-MS analyses. However, the sensitivity of the instrument (relevant molecules are detectable at ppb levels) is such that even a small amount of chemical alteration to the irradiated portion of the sample should be detectable after dilution by portions from the non-irradiated areas (at most 50% of the sample surface).

RESULTS AND DISCUSSION

The Raman analyses of both organic compounds were performed through the bottom of the borosilicate glass vials. As a result, a Raman band at around 450 cm⁻¹, assigned to Si-O-Si bending, was observed in all Raman spectra, especially for palmitic acid. A representative Raman spectrum

obtained from an empty borosilicate vial is shown in Figure 2. The weak peak around 800 cm^{-1} may represent O-Si-O symmetric stretching (Manghnani *et al.*, 2011).

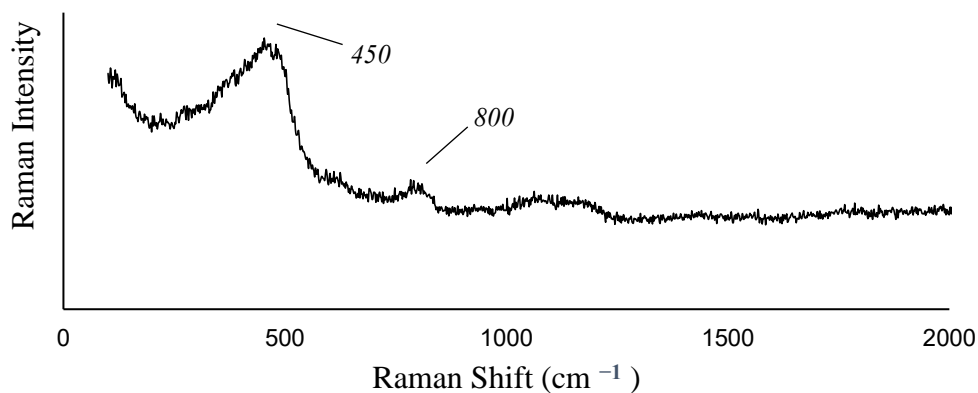


Figure 2. Raman spectrum of an empty borosilicate glass vial

Palmitic acid

Raman spectra of palmitic acid compared prior to and after irradiation indicate no observable chemical changes (Figure 3). Both spectra are similar in terms of the location of Raman bands. Several peaks, identified in Figure 3, are present in both sets of spectra. These include a C-H₂ twist located at around 1300 cm^{-1} , a C-H₂ bend at around 1440 cm^{-1} , C-H₂ symmetric and asymmetric stretches between $2800\text{-}3000\text{ cm}^{-1}$, and C-C stretching around $1050\text{-}1200\text{ cm}^{-1}$ (Czamara *et al.*, 2014; Wu *et al.*, 2011). The bands attributed to Si-O-Si bending around 450 cm^{-1} from the glass vial are also present in both sets of spectra.

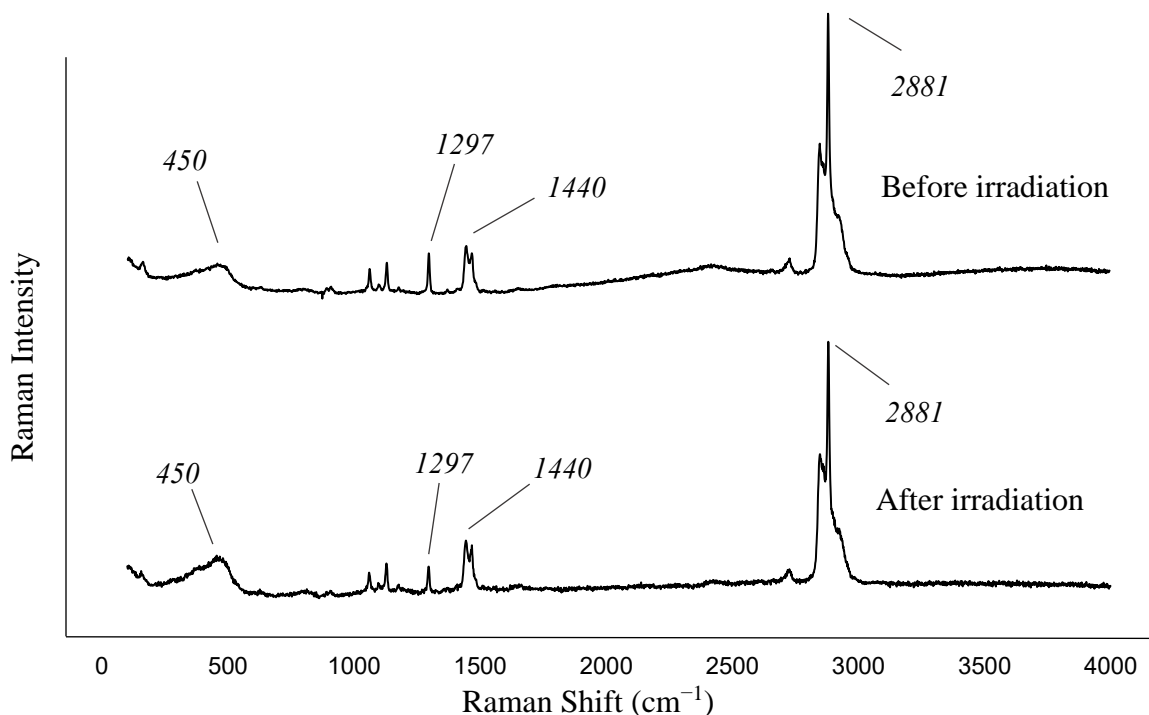


Figure 3. Observed Raman spectra of palmitic acid before (upper panel), and after irradiation (lower panel) with excitation at 532 nm. Major peaks are labeled by energy of vibration. The vibrational modes observed for both samples are identical, indicating no observable chemical alteration.

The GC-MS chromatograph shown in Figure 4 demonstrates a peak at 18.80 min (retention time) for irradiated palmitic acid, corresponding to the molecular ion signature M⁺, 256.27 m/z in the mass spectrum. Other peaks in the mass spectrum correspond to ionized fragments of palmitic acid.

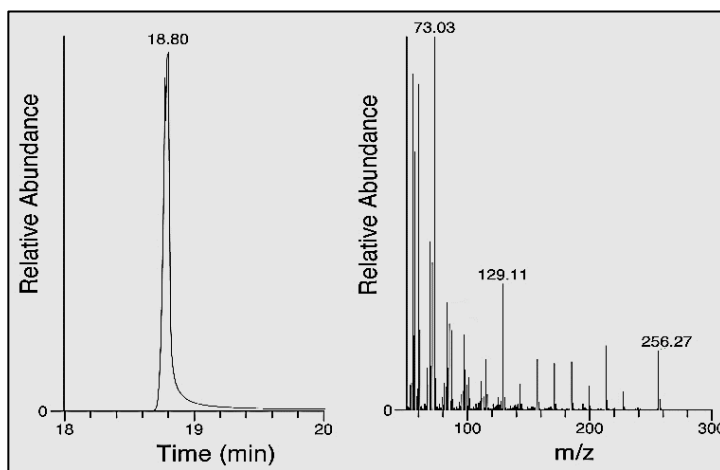


Figure 4. Only one constituent is indicated by the results of GC-MS analysis of irradiated palmitic acid. Palmitic acid corresponds to the peak with an 18.80 min retention time, and the molecular ion signature M⁺, 256.27 m/z.

Pyrene

The Raman spectrum of pyrene after X-ray exposure (Figure 5) indicates pyrene was the main component in the sample. The Raman spectrum of pyrene after irradiation was identical to non-irradiated (pure) examples reported in the literature (e.g. Alajtal *et al.*, 2015; Shinohara *et al.*, 1998). The peaks at 406 and 592 cm^{-1} are associated with skeletal bending of pyrene, and the band at around 1066 cm^{-1} corresponds to C-H bending. Ring stretching is observed around 1406 cm^{-1} and C-C stretching bands are seen around 1590 -1629 cm^{-1} (Xie *et al.*, 2010; Alajtal *et al.*, 2015). No evidence of X-ray induced chemical alteration was observed in the Raman spectrum.

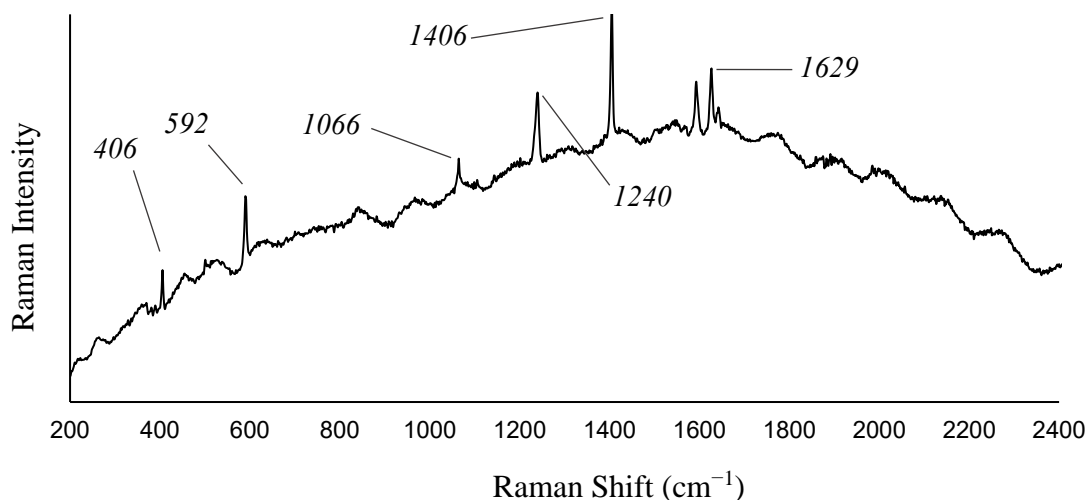


Figure 5. Raman spectrum of irradiated pyrene with excitation at 532 nm. No evidence of X-ray-induced alteration was observed in the Raman spectrum. The broad underlying band in the spectrum is due to fluorescence. The small glass peak at around 450 cm^{-1} is not clearly seen.

The GC-MS spectra and chromatograms of pyrene before and after irradiation are illustrated in Figure 6. Pyrene corresponds to the 202.1 m/z molecular ion that is observed in both spectra. Other peaks correspond to ionized fragments of pyrene. The broader peak after irradiation compared to before irradiation in Figure 6 is most likely due to the higher concentration of pyrene injected on the GC-MS column.

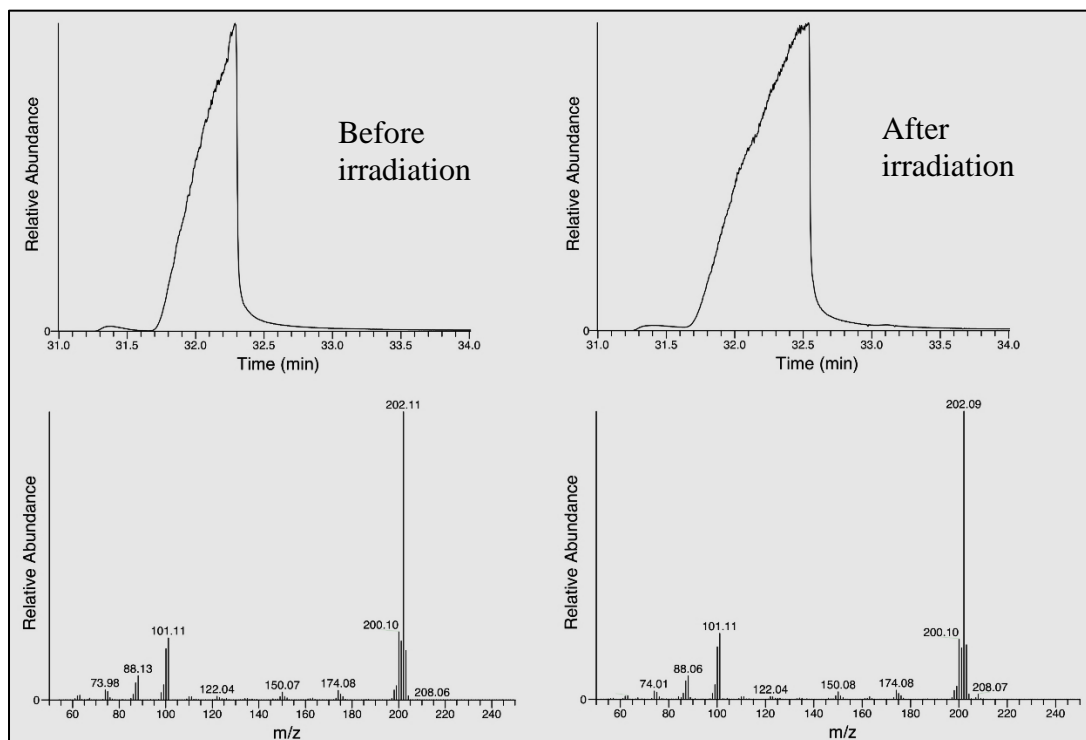
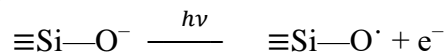


Figure 6. GC-MC analysis of non-irradiated (left) and irradiated (right) pyrene after 90-hour exposure to 50 kV X-ray source. Both GC-MS spectra indicate the presence of one constituent (pyrene). No evidence of X-ray induced chemical alteration was observed.

Ultimately, the combined results of GC- MS and Raman analyses for both organic samples indicate that no significant chemical alteration of the samples occurred.

Formation of Color Centers in Borosilicate vials

Ionizing radiation can activate color centers as a result of the displacement of lattice atoms or by producing electron hole pairs in the glass. The X-ray irradiated area then forms an absorption band in the visible spectra (cf. Zhang *et al.*, 2007). In this experiment, a yellow-brown color was seen in glass vials after exposure to the X-ray beam. We suggest the most likely mechanism for the absorption band formation is the production of a non-bridging oxygen hole center (right side) from non-bridging oxygen (left side).



The released electron in the right side of this reaction can be trapped in the glass network, causing a visible absorption band (Kadono *et al.*, 2009; Ehrt and Vogel, 1992). The color change caused by ionizing radiation in such samples is typically reversible after exposure to heat (Marshal *al.*, 1997). If the irradiated glass is heated, the movement of atoms will increase, leading to the rearrangement of the silicon/boron atoms in a process called annealing (El-Khashen, 2012). In the current study, the authors irradiated an empty glass vial and reversed the color change by thermally annealing the vial at 350 °C for 2 hours.

CONCLUSION

The effects of X-ray irradiation generated by a micro-focus XRF instrument on organic materials commonly reported from the terrestrial rock record were assessed using Raman spectroscopy and GC-MS. Pyrene and palmitic acid were deposited in borosilicate glass vials and irradiated under a Bruker ARTAX instrument operated at 50 kV/0.6 mA. A distinctive color change was observed in the glass vials after several hours of irradiation. However, no observable chemical alteration in the samples was detected by either Raman spectroscopy or GC-MS. The Raman spectra of palmitic acid and pyrene before and after irradiation were identical/comparable to examples reported in the literature. GC-MS analysis also indicated no chemical change in the irradiated samples. The reversible color change observed in the glass vials was likely caused by activation of color centers in the structure of the borosilicate glass vials. We conclude that micro-XRF irradiation did not induce photochemical alteration in palmitic acid or pyrene under our experimental conditions, but note that our study was of limited scope. Our use of glass vials to contain the sample during measurement in a laboratory setting is not directly analogous to the application of such instruments in the field. In the real world, micro-XRF instruments operate at a variety of energy levels, and organic molecules are typically trapped within minerals of widely varying elemental composition. Although this evaluation was not directly analogous to actual application in the field, our study provides an example of experimental procedures for this type of evaluation that may be of use to future studies.

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