



## COMPARATIVE STUDY OF TOXIC SPECIATION: $[\text{Cr}^{3+}/\text{Cr}^{6+}]$ AND $[\text{As}^{3+}/\text{As}^{5+}]$ IN CRUDE OIL AND ASPHALTENE DERIVATIVE USING HYPHENATED LIQUID CHROMATOGRAPHY AND PLASMA QUADRUPOLE MASS SPECTROMETRY

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### ABSTRACT

A comparative study of toxic speciation in crude oil and asphaltenes has not been previously reported and contributes to knowledge in fuels research. Crude oil is used on land farms; and asphaltenes are converted to asphalt for constructing roads. Toxic components in these substances could, therefore, pose a distinct threat to the environment. In this study, asphaltene fractions were isolated from crude oil samples via soxhlet extraction. The native crude oil and abstracted asphaltenes were then subjected to investigation for toxicity using an ultra-sensitive hyphenated facility. A Perkin Elmer SCIEX DRC-e ICP-MS was coupled with a liquid chromatographic system (HPLC) for simultaneous detection and convenient resolution of all four species. Stationary and mobile phases were prepared according to standard protocol. The elution rate was 1.2 mL/min; and corresponding retention times (min) were:  $\text{As}^{3+}$ : 1.61;  $\text{As}^{5+}$ : 4.06;  $\text{Cr}^{3+}$ : 3.71; and  $\text{Cr}^{6+}$ : 5.80. The system possesses a built-in dynamic reaction cell (DRC) to enhance mass detection. The oxygen flow in the DRC was controlled to give a rate of ~0.4 mL/min. Following mass detection,  $m/z$  values of 52 characterized the  $\text{Cr}^{3+}/\text{Cr}^{6+}$  species; while the arsenic components were detected as adduct ions,  $\text{AsO}^+$ ,  $m/z$ , 91, after coalescing with oxygen. The levels of the species of interest were compared for crude oil and asphaltenes and the data revealed a preponderance of the detected species in the asphaltene phase. The feasibility of applying the experimental data in “fingerprinting” studies was examined; and the impact of our study in terms of environmental protection and sustainable development is discussed.

**Keywords:**  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$ ; HPLC/ICP-MS; crude oil, asphaltenes.

### INTRODUCTION

Limited research has been conducted on toxic speciation in crude oil and its derivatives. Characterization of noxious chemical species  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  in fuels represents an uncharted area of research, and from this perspective our work is seminal and breaks new ground. Both crude oil and asphaltenes have practical uses and are used on land farms and for construction purposes (Nalwaya *et al.*, 1999; Carrier *et al.*, 2000; Spiecker *et al.*, 2003). Asphaltenes are an essential component of crude oil normally isolated from the virgin fuel via soxhlet extraction (Spiecker *et al.*, 2003). They contain bound metal and metalloid components within their porphyrin-like structures that originate largely from source rock, mineral matter and formation water (Carrier *et al.*, 2000). The waste sediment of crude oil, oil-sludge, is usually deployed on oil-sludge farms to enrich soil. Asphaltenes on the other hand are used to build roads, pavements and roofing materials. Toxic chemical species present in these materials are thus released to the environment and could

pose a hazard if present at elevated levels (Pillay *et al.*, 2014). The primary objective of this investigation was to characterize noxious trace chemical species  $\text{Cr}^{6+}/\text{Cr}^{3+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  in crude oil and its asphaltene derivative.

There is a growing need to promote sustainable living, where it is acknowledged that any form of environmental risk could be detrimental to the ideology of sustainability (Robinson, 1993; Shearman, 1990). There is general support, therefore, to expose all possible threats to environmental safety especially from energy sources such as fossil fuels and biofuels. It is well known that the chemical species of interest are toxic at elevated levels (Pillay *et al.*, 2014), prompting an increasing demand for accurate information linked to potential environmental hazards of this nature. Measurement of the species of interest was undertaken by a powerful hyphenated facility combining high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The system has a built-in dynamic reaction cell (DRC) for superior results, and possesses the distinct

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capacity of isolating all four species [ $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$ ] concurrently in a single run followed by high resolution mass detection (Li *et al.*, 2015). In the HPLC unit retention times interfere with the process but complete separation is attained when the species in question are conveyed via quadrupole selection to the mass detector (Li *et al.*, 2015). The system is ultra-sensitive (ng/L) and of outstanding capability compared to most contemporary analytical techniques (Peisach *et al.*, 1994; Abbu *et al.*, 2000; Peisach *et al.*, 1994a; Naqvi *et al.*, 2014; Feist and Mikula, 2014). The feasibility of grouping asphaltene samples on the basis of chemical speciation for use in “fingerprinting” studies was explored.

## MATERIALS AND METHODS

### Soxhlet Extraction/Liquid Chromatography

Crude oil samples were mixed with n-heptane and allowed to ‘age’ for three days. After the aging period

asphaltene fractions were extracted via multiple soxhlet distillation (Fig. 1), and purified by refluxing with n-heptane (Spiecker *et al.*, 2003). The solubility parameters and density measurements ( $\sim 1.13 - 1.20\text{g/mL}$ ) confirmed the authenticity of the abstracted asphaltene phase (Carrier *et al.*, 2000). A Perkin Elmer instrument (Fig. 2) was employed for isolation of all four species -  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$ . The stationary phase was composed of deactivated silica, pore size:  $5\ \mu\text{m}$ , and consisted of a 150 mm column (C8) - internal diameter, 4.6 mm. The mobile phase was a homogeneous solution of pH 7.2 (adjusted with dilute aqueous  $\text{HNO}_3/\text{NH}_4\text{OH}$ ), composed of  $1 \times 10^{-3}\text{M}$  TBAH,  $0.5 \times 10^{-3}\text{M}$  EDTA and 5% aqueous methanol. The system is equipped to handle low sample volumes ( $50\ \mu\text{L}$ ) with an optimal elution rate of 1.2 mL/min. Details for preparing the mobile and stationary phases appear in Perkin Elmer reports on the subject (Neubauer *et al.*, 2004) and were adopted for purposes of optimization. The system was calibrated using certified standards obtained from VHG Labs (USA).



Fig. 1. Multiple Soxhlet Unit.

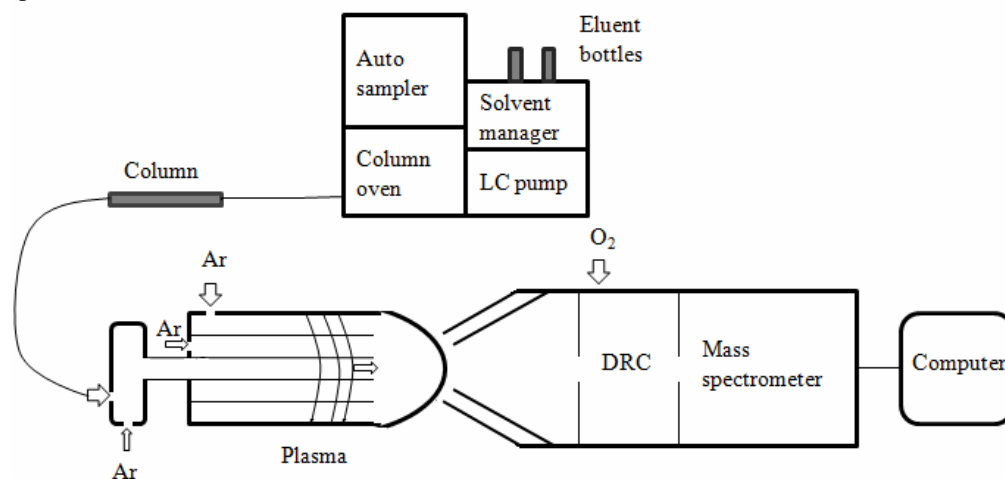


Fig. 2. Schematic of the HPLC-DRC-ICP-MS system.

### Dynamic Reaction Cell (DRC) / Plasma Mass Spectrometry

Mass separation of the isolated species was attained with a quadrupole selector in a Perkin Elmer SCIEX DRC-e ICP-MS fitted with a dynamic reaction cell (DRC) (Fig. 2). The nebulizer gas flow was 0.80 L/min and the oxygen flow in the DRC was 0.4 mL/min. The main purpose of the DRC was to reduce interference by interaction of the eluted species with the oxygen flow gas.  $\text{Cr}^+$  was detected at  $m/z$  52 ( $m/z$  is the mass-to-charge ratio); and  $\text{As}^+$  was detected as an adduct ion,  $\text{AsO}^+$ ,  $m/z$  91, after reacting with oxygen. The ICP-MS was calibrated with certified standards (Elan 6100; 10.00 ppb per element); and repeatability studies produced a spread in the data of <10% indicating that the system's performance was acceptable (Table 1). Drift in instrumental performance was corrected by use of an internal standard. Samples and

standards were all made up in aqueous solution consistent with the mobile phase. The software adjusted random matrix effects.

## RESULTS AND DISCUSSION

### Collision Cell/Retention Times

The notion of combining a collision cell with HPLC/plasma mass spectrometry is to resolve the overlap in retention times that are encountered when all four species of interest are simultaneously eluted from a conventional chromatographic column. Interfering retention times make regular analysis almost impossible and conventional liquid chromatographic techniques fail to conveniently distinguish all four species in a single run. In the present study the recorded retention times (min) were as follows:  $\text{As}^{3+}$ : 1.61;  $\text{As}^{5+}$ : 4.06;  $\text{Cr}^{3+}$ : 3.71;  $\text{Cr}^{6+}$ :

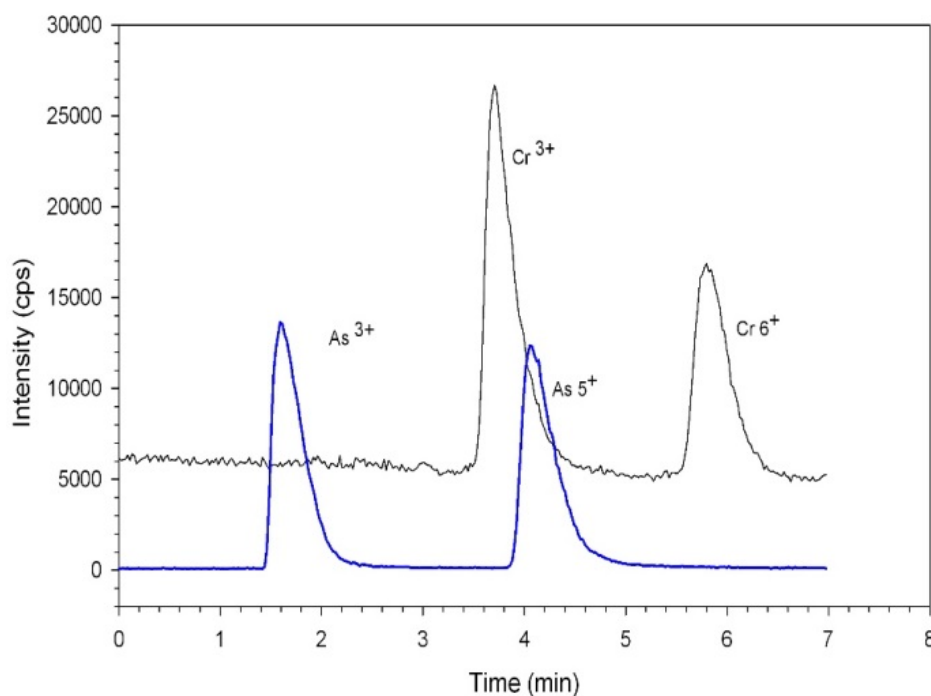


Fig. 3. Typical HPLC chromatograms showing retention times for  $\text{As}^{3+}$  / $\text{As}^{5+}$  and  $\text{Cr}^{3+}$  / $\text{Cr}^{6+}$

Table 1. Repeatability test of the ICP-MS for various elements ( $\mu\text{g/L}$ ) in an aqueous standard showing the range and % spread.

Element	Trial #1	Trial #2	Trial #3	Range	%Spread
V	10.01	9.36	9.82	0.65	6.9
Cr	9.51	10.04	10.07	0.56	5.9
Co	10.54	9.82	10.12	0.72	7.3
Ni	10.08	9.46	9.56	0.62	6.6
As	9.82	10.21	9.74	0.47	4.8
Mo	9.89	9.14	9.72	0.75	8.2
Cd	9.51	10.06	10.09	0.58	6.1
Tl	9.59	9.65	10.21	0.62	6.5
U	10.08	9.65	10.12	0.47	4.9

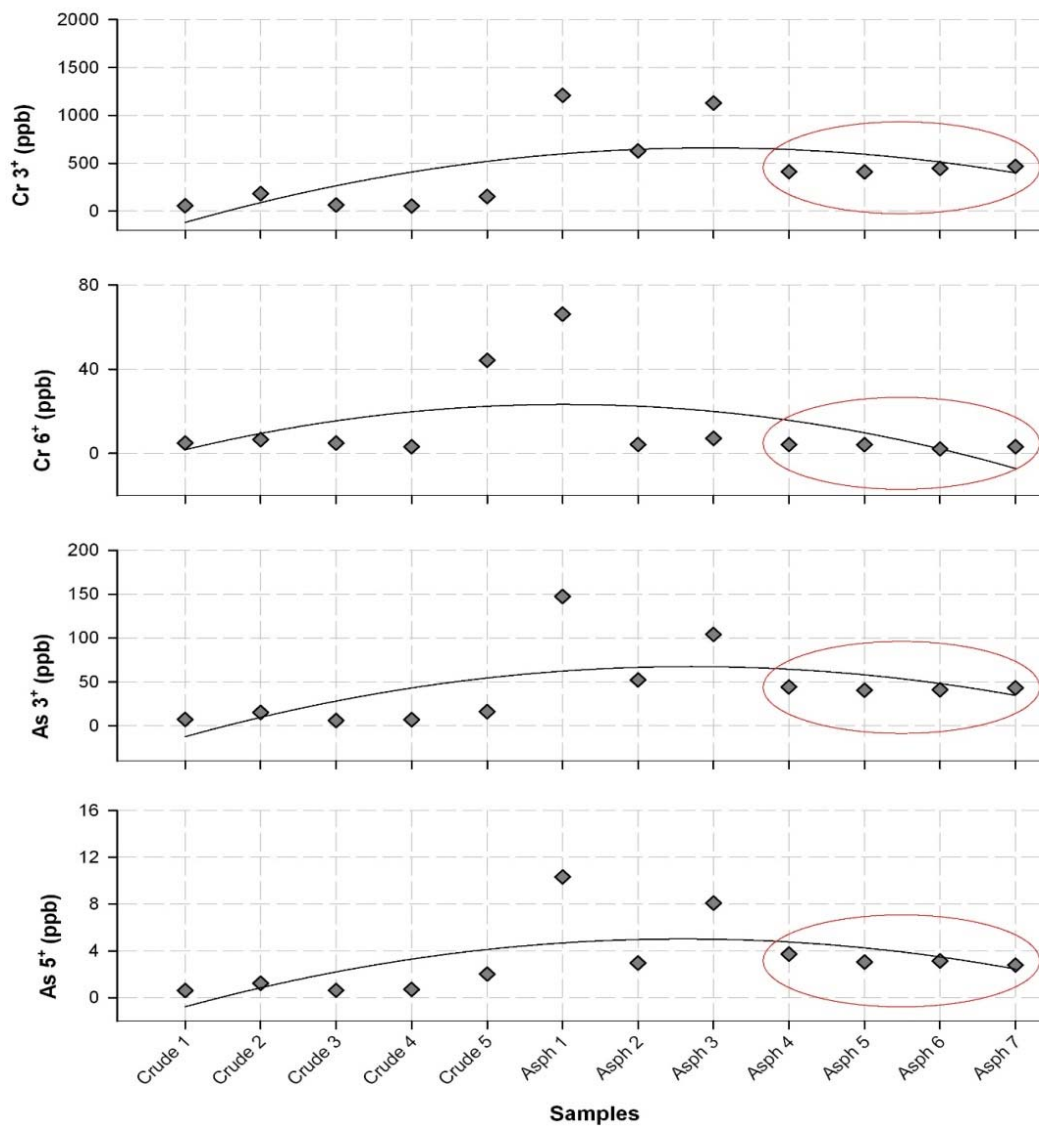


Fig. 4. Levels of chemical species of interest showing “fingerprints”.

5.80. Inspection of figure 3 clearly indicates that  $As^{5+}$  overlaps with  $Cr^{3+}$ ; and as it tails off the tail-end of its peak structure could interfere with  $Cr^{6+}$ . Of significance is that asymmetry in peak structure has to be optimized for favorable results, accomplished by adjusting the instrument to standard protocol (Dolan, 2003). The proximity of the chromatograms displayed in figure 3 reveals that deconvoluting the data to provide a meaningful analysis would be futile, and the only possible solution of attaining distinct resolution would be to transport the eluted species to an ICP equipped with a dynamic reaction cell (DRC). It is necessary to emphasize that the technique employed here using the DRC (or collision cell) for ultimate mass detection is a unique way of clearly differentiating between all four species. The cell itself works by utilizing a flow of oxygen to either dissociate interfering species or to coalesce with the

species of interest for suitable resolution. For example  $ArC^+$  (present at  $m/z$  52) created within the plasma itself interferes with  $Cr^+$  at  $m/z$  52. Oxygen gas in the DRC dissociates  $ArC^+$  and thus suppresses the interference. On the other hand,  $As^+$  (at  $m/z$  75) binds to oxygen to form a complex adduct, and is detected at  $m/z$  91, thus overcoming interference from instrumental species  $ArCl^+$  and  $CaCl^+$  that also occur at  $m/z$  75. A quadrupole selector built into the system steers the charged species to a mass detector for clear resolution and easy detection. The system is quick and can cope with high sample throughput with high precision.

#### Asphaltene Derivative

Direct comparison of the levels of the species of interest between crude oil and asphaltene extracts shows distinct differences (Fig. 4). The data denote a definite trend and,

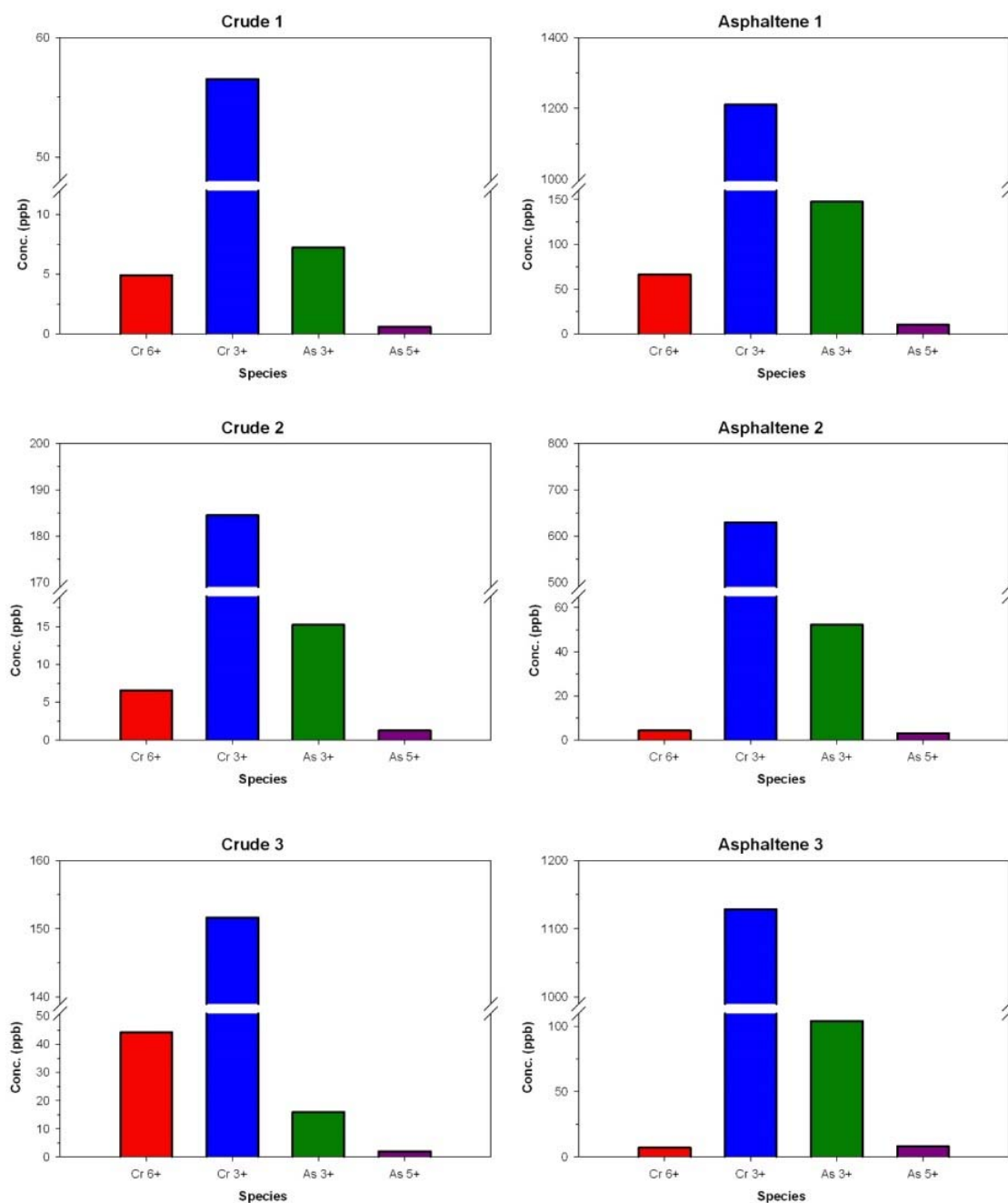


Fig. 5. Comparison between crude oil levels and corresponding asphaltene levels for selected samples.

apart from  $\text{Cr}^{6+}$ , suggest that the remaining species tend to accrue largely in the abstracted asphaltene fractions. The precise interpretation of this phenomenon is not clear but it would seem that the explanation could possibly lie in the catagenesis of asphaltenes (Speight *et al.*, 2004). The origins of asphaltenes lie in source rock and mineral matter (Speight *et al.*, 2004) and the formative stages represent an agglomeration of a wide range of heavy hydrocarbons to form the complex asphaltene fraction as we know it. Fusion of hydrocarbons leads to coalescing of metal and metalloids species within porphyrin-like structures. The experimental data convey the notion that the metal and metalloids species in question tend to amass

in the asphaltene conglomerate, thus making it unusually rich in trace toxic species.

In crude oil the ranges were as follows:  $\text{Cr}^{3+}$ : 50-200 mg/L;  $\text{Cr}^{6+}$ : 3-45 mg/L;  $\text{As}^{3+}$ : 6-16 mg/L;  $\text{As}^{5+}$ : 0.5-2 mg/L. In asphaltenes the ranges were:  $\text{Cr}^{3+}$ : 400-1200 mg/L;  $\text{Cr}^{6+}$ : 2-70 mg/L;  $\text{As}^{3+}$ : 40-150 mg/L;  $\text{As}^{5+}$ : 2-12 mg/L. In all cases differences in levels up to a factor of about 20 were encountered between the crude oil and asphaltene fractions. These differences are appreciable and support the view that metal/metalloid gravitation in the asphaltene phase is strongly linked to the developmental phases in asphaltene formation (Speight,

2004). The accumulation and preponderance of these species in asphaltenes have clear implications related to environmental safety and protection, discussed below. It is interesting to note the curvilinear nature of the plots in figure 4 - pronounced for  $\text{Cr}^{6+}$ , which, in the absence of outliers could delineate a linear regression showing little or no difference between the crude oil and asphaltene results (Fig 4). This particular phenomenon could be linked to the chemistry of porphyrins and reconciled with the theory that to satisfy energy stability and the rigors of stereochemistry the porphyrin-like asphaltenes find difficulty in complexing with highly charged metal species (Speight, 2004). These species are thus drawn into the crude oil layer.

### “Fingerprinting”/Provenancing

An interesting feature of the results is that it may be possible to use the asphaltene data for “fingerprinting” studies, which could be linked to mapping or provenancing. The feasibility that asphaltenes could be grouped on the basis of their trace toxic content was thus explored. Figure 4 depicts the levels of all samples and close inspection shows that some samples (encircled) can be grouped on the basis of the concentrations of the trace chemical species of interest. This was an unexpected development, but useful information, to encourage further research in this direction. The clusters denote oil wells that could originate from the same oilfield. The results in figure 4 provide convincing evidence that all four species could serve as useful candidates for application in provenancing studies – and the groupings are distinct with no ambiguity. Provenancing (Punyadeera *et al.*, 1997) is a subject that essentially links samples or specimens to specific areas or provinces. It has been used successfully with archaeological artefacts and the unique development that has emerged from this study is that all four species could serve as “tracers” or “markers” to identify or “fingerprint” wells in a demarcated area. The interesting point that arises from figure 4 is that all four species act in mutual consonance to confirm the provenance of the encircled samples. This revelation would not have come to light were it not for the pursuit of this toxicity study. Grouping or clustering using macro-elements such as sodium or magnesium would probably fail because they are not as discrete as the chemical species in question. The subject is certainly worthy of further investigation and could make a useful contribution to the fuels industry.

### Toxicity/Environmental implications

Reports by other authors (Duyck *et al.*, 2007; Hardaway *et al.*, 2004) have suggested that heavy metals in crude oil (and asphaltenes) could pose a distinct hazard in various environmental surroundings. Aquatic toxicity is an interesting example, and oil spills are known to be detrimental to marine organisms and algae. On the other hand oily sludge deployed on land farms (Taha *et al.*, 2001) could indirectly pollute overhead streams and water supplies through agricultural runoff. In addition crude oil

dumped in land-fills has the potential of ultimately infiltrating the water table and contaminating subterranean aquatic reserves, thus posing a secondary threat to human health (Pillay *et al.*, 2010).

The data in the foregoing plots (Fig. 4) suggest that the species tend to settle in the asphaltene phase. Fig 5 highlights three selected crude oil samples and corresponding asphaltene extracts to emphasize this point. The general trend in figure 5 is clear – toxic levels are considerably higher in the correlating asphaltene samples. The guideline for admissible levels in industrial water and effluent should not exceed  $5\mu\text{g/L}$  (5 ppb) (Pillay *et al.*, 2014; Neubauer *et al.*, 2004). From the perspective of environmental protection and safety the levels of the species in question are elevated. The results in figure 5 (and Fig. 4) demonstrate that all concentrations widely exceed standard permissible levels, and are especially pronounced in the asphaltene fractions. This underlines the severity of the potential threat posed to the ecosystem when asphaltenes are released to the environment. Asphaltenes are usually converted to materials to construct roads, pavements, paving for shopping malls and car parks. They are also deployed as roofing materials and curing agents. As aforementioned, elevated levels of toxins in asphaltenes could be hazardous especially when these toxins are leached and find their way to the water table. In arid countries where aquatic resources are scarce the threat is tangible. In particular,  $\text{Cr}^{6+}$  displays higher toxicity than  $\text{Cr}^{3+}$ ; and  $\text{As}^{3+}$  is more noxious than  $\text{As}^{5+}$  (Eary and Rai, 1987; Katz and Salem, 1993; Korte and Fernando, 1991). Notable in figure 5 is that  $\text{Cr}^{6+}$  levels range from 4-70 mg/L, which are elevated by more than three orders of magnitude above environmentally permissible levels.  $\text{As}^{3+}$  display levels that range between 40-150 mg/L, which are equally abnormal. Sustainable development repels any form of activity that tends to threaten natural resources (Robinson, 1993; Shearman, 1990). These concentrations are acute and call for demetallization procedures to “detoxify” asphaltenes in the interest of environmental protection and sustainability.

### Impact of the study

The determination of trace chemical species  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  in asphaltenes and crude oil has not been previously documented and constitutes original research. Successful application of the instrumental technique itself is a notable achievement for the simple reason that all four species can be detected simultaneously in a single run – a feat not within the capability of any other contemporary technique. Isolating the eluted species from crude oil and asphaltenes in an HPLC system and transporting them via a dynamic reaction cell through a quadrupole selector for mass differentiation is a novel development in fuel studies. An unusual feature was the feasibility of using the trace toxic species for purposes of “fingerprinting” and provenancing. As mentioned above this was an unforeseen, but welcome, development. We



found that all four species of interest in asphaltenes could serve as potential markers or “fingerprints” to identify wells in an oilfield. This could be a subject for further detailed development where provenancing of this nature could possibly be based on parameters such as the  $\text{Cr}^{3+}$ :  $\text{As}^{3+}$  ratio (for example). Perhaps the most striking impact of this study was the elevated levels of the toxic species of interest. It is known that  $\text{Cr}^{6+}$  and  $\text{As}^{3+}$  at elevated levels are responsible for disorders in the human body (Eary and Rai, 1987; Katz and Salem, 1993; Korte and Fernando, 1991). Unwanted toxins find their way to aquatic sources that are used for domestic purposes. These elevated concentrations are detrimental and pose a definite threat to the environment. The potential threat from disposal of crude oil is equally concerning and should not be minimized (Pillay *et al.*, 2010). We, therefore, recommend removal of metals/metalloids from asphaltenes and crude oil prior to their use in the environment. In this respect, cost-benefit considerations and logistics for such demetallisation procedures are areas that will require comprehensive evaluation.

## CONCLUSION

The use of trace toxic species in asphaltenes:  $\text{Cr}^{3+}/\text{Cr}^{6+}$  and  $\text{As}^{3+}/\text{As}^{5+}$  to provide an insight into provenancing or “fingerprinting” studies is a novel development and should be pursued further. We found that the levels of the species in question were abnormally high in both crude oil and asphaltene samples, which necessitates chemical treatment for “detoxification.” The hyphenated technique employed in this study (HPLC-DRC-ICP-MS) is unique with multiple features, displaying high performance and ultra-sensitivity. The study could be extended to other chemical species in fuels, such as  $\text{Se}^{4+}/\text{Se}^{6+}$ . Our work makes a distinct contribution to fuels research and environmental science and more data are expected to be obtained in a follow-up study.

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