BORSA SIMP 2011 PER UN SOGGIORNO DI STUDIO ALL'ESTERO: RELAZIONE SULL'ATTIVITA' SVOLTA

SURFACE REACTIVITY OF FIBROUS AMPHIBOLES OF ENVIRONMENTAL AND HEALTH INTEREST

ALESSANDRO PACELLA
Dipartimento di Scienze della Terra, Università di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma, Italy

INTRODUCTION

It is well known that exposure to asbestos fibres, both for occupational and non-occupational reasons, is linked to numerous health problems and respiratory diseases (Murray, 1990). Epidemiological data show that moderate exposure to chrysotile causes a very low health risk if compared to amphibole asbestos. These findings may be linked to the observed largely different solubility of asbestos fibres in the simulated lung and gastric fluids: very high for chrysotile and significantly lower for amphibole asbestos (Van Oss *et al*., 1999; Oze & Solt, 2010). In addition, the iron content of asbestos fibres and its chemical state are believed to play a crucial role in the toxicity control, since iron can catalyse the production of reactive oxygen species (ROS) and determine the release of free radicals (*e.g*., the hydroxyl radical HO°). These are responsible for the damage of biological organic molecules, *e.g*., DNA, lipids and proteins (Kane, 1996; Fubini & Otero Aréan, 1999; Kamp & Weitzman, 1999; Robledo & Mossman, 1999). The mechanism generally accepted for ROS production is the iron-catalysed Haber-Weiss cycle (Fubini & Otero Aréan, 1999):

$$\text{Fe (III)} + \text{reductant} \leftrightarrows \text{Fe (II)} + \text{oxidized reductant}$$
$$\text{Fe (II)} + O_2 \leftrightarrows \text{Fe (III)} + O_2^{\circ -}$$
$$O_2^{\circ -} + 2H^+ + e^- \leftrightarrows H_2O_2$$
$$\text{Fe (II)} + H_2O_2 \leftrightarrows \text{Fe (III)} + OH^- + HO^{\circ}$$

In addition to the wide asbestos literature, recent epidemiological studies revealed some cases of environmental contamination of non-regulated fibrous amphiboles. In Italy, a study on the geographical distribution of mortality from malignant pleural mesothelioma evidenced a high and unusual cluster of malignant mesothelioma cases among inhabitants of Biancavilla, a town located in Sicily on the southwestern side of Etnean volcanic area (Di Paola *et al*. 1996). Epidemiologic and environmental surveys in Biancavilla showed no asbestos exposure either from occupational activities or from the use of manufactured products. However, it was documented that the local incohesive volcanic materials were usually quarried for building industry and road paving (Paoletti *et al*., 2000). Mineralogical investigation detected prismatic, acicular and fibrous amphiboles in the quarry material. The amphiboles were characterized in detail and proved to be compositionally heterogeneous, containing tremolite, winchite and the new amphibole end-member fluoro-edenite (Gianfagna *et al*., 2003; Mazziotti-Tagliani *et al*., 2009; Andreozzi *et al*., 2009). It was suggested that the unusual cluster of mesothelioma cases could be caused by the exposure to such fibrous amphiboles (Comba *et al*., 2003). As a matter of fact, the fibres from Biancavilla showed high carcinogenicity in intraperitoneal injection experiments with rats (Soffritti *et al*., 2004). In addition, recent studies *in vitro* revealed that their toxicity is strongly related to their chemical composition, with particular relevance for Fe content and its oxidation state (Cardile *et al*., 2004; Pugnaloni *et al*., 2007). As a consequence of previous studies, the Biancavilla quarry was closed and the Biancavilla area has been included in the National Priorities List of sites needing remediation. In spite of this, fibrous fluoro-edenite is still not regulated as asbestos.

Another well-known case of occurrence of non-regulated fibrous amphiboles is that of Libby (Montana, USA). In the Libby area an elevated incidence of lung-cancer and mesothelioma was recognised within the local miners and millers, which worked in vermiculite mine operated from 1923 to 1990 for the local building industry (McDonald *et al*., 1986; Wilye & Verkouteren, 2000). The studies of Wilye and Verkouteren (2000) and Gunter *et al.* (2001, 2003) demonstrated the presence in the vermiculitic deposits of fibrous amphiboles, with composition ranging from richterite to winchite. As a consequence, the environmental survey finally related the workers' disease to the occurrence of asbestiform richterite and winchite (both not regulated as asbestos).

Although a wide literature exists on asbestos minerals, in the present work chemical reactivity of fibrous amphiboles not regulated as asbestos (two samples of fibrous amphibole from Biancavilla, one sample of fibrous amphibole from Libby) was investigated for the first time. Possible relations between surface chemical reactivity and surface Fe features (*i.e*., amount, oxidation state) as well as bulk Fe crystal chemistry of fibrous amphiboles have been explored. This study, financially supported by the SIMP scholarship, was carried out during the permanence at the Laboratoire de Réactivité de Surface of the University of Paris (Paris VI).

PERFORMED RESEARCH

Materials

The fibrous amphiboles here investigated come from the volcanic products outcropping around Biancavilla town, Sicily, Italy (sample 1 and sample 4, as named in Mazziotti-Tagliani *et al*., 2009 and Andreozzi *et al*., 2009), and from the vermiculite mine of Libby, Montana, USA (hereafter named Libby).

Surface reactivity investigation: HO° radical production

The study of surface reactivity of our samples was performed at the Laboratoire de Réactivité de Surface of the University of Paris (Paris VI), laboratory pioneer in Europe for the surface reactivity and toxic chemistry studies.

The capability of the fibrous samples to release free radicals was evaluated by measuring the HO° radical production in presence of H_2O_2 via Fenton reaction. All tests were performed in a reactor at 37 °C, in absence of light. The reaction mixture contained 25 mg of sample (previously ground for 1 minute), 0.5 ml of sodium phosphate buffer (Na_2HPO_4-NaH_2PO_4, Sigma, 99% in purity) at *p*H = 7.4 (0.5 M), 1 ml of aqueous buffered solution of DMPO (5.5-dimethyl-1-pyrroline-N-oxide, Aldrich, 97% in purity) as a radical trapping agent (0.1 M), and 0.5 ml of buffered H_2O_2 (0.6%/vol) obtained after dilution of commercial solution (Prolabo, Normapur). The reactor was placed on a swinging table in order to ensure the homogeneity of the suspension. After 5 and 30 minutes of incubation time, fractions of the suspension were filtered (cellulose acetate filter, 0.22 µm) and the filtrate was transferred to a flat quartz cell for the detection of the radical adduct [DMPO, HO]° by Electron Paramagnetic Resonance (EPR) spectroscopy. Control measurements were realised in parallel using blank solutions not containing fibrous samples measured in the same experimental conditions.

The EPR spectra of [DMPO, HO]° were obtained at room temperature using a Brucker ESP-300E spectrometer. Analytical conditions were: magnetic field 3440 G, frequency field 9.65 GHz, power 10 mW, frequency modulation 100 KHz, modulation amplitude 3.24 G and gain of 5*104, acquisition time 84.92 ms.

RESULTS

Results of EPR measurements of the production of (DMPO, HO)° radical adduct are reported as the average of the signal collected after 5 and 30 minutes of incubation time, subtracted by the value obtained for the control. Production of HO° radicals by fibrous sample from Libby is higher than that of fibrous samples from Biancavilla, which in turn did not show significant differences each other (Fig. 1). Moreover, the reactivity of the fibrous samples studied in this work, though markedly lower than that of UICC crocidolite, which is well known to be extremely reactive, resulted to be comparable with that of fibrous tremolite (Pacella *et al*., 2010; 2012). The production of HO° radicals of the investigated fibrous samples did not correlate very well with total Fe

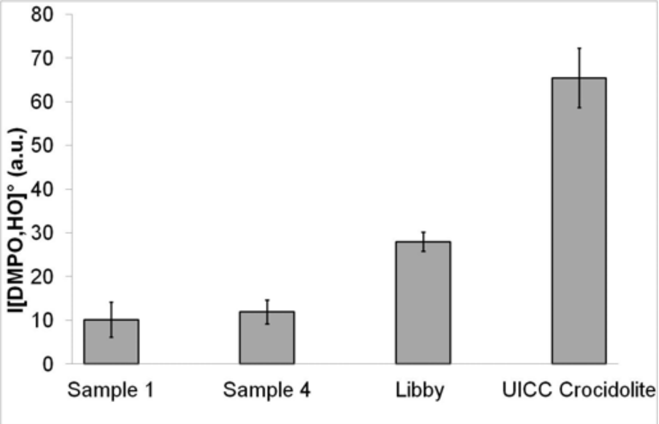

Fig. 1 - Production of (DMPO, HO)° radical adduct for the investigated samples, measured by EPR in presence of H_2O_2 in buffered phosphate medium (*p*H 7.4). UICC crocidolite (DMPO, HO)° is taken from Pacella *et al*. (2012). The intensity is reported in arbitrary unit. Error bars correspond to ±2σ.

content (data not shown), but fairly correlates, with the only exception of the sample from Libby, with the Fe (II) located at M(1)+M(2) sites in the amphibole structure (Fig. 2a). The amphibole structure, shown in Fig. 2a, consists of a double-chain of corner sharing tetrahedral, denoted by T, and a strip of edge-sharing octahedral, denoted by M. There are two distinct sites T(1) and T(2), occupied by cations tetrahedrically coordinated, and three distinct types of octahedra designated as M(1), M(2) and M(3). The double chain of tetrahedra and the strip of octahedra are linked by the M(4) site. According to the hypothesis already formulated for fibrous tremolite, iron in M(1) and M(2) sites is the most exposed and has the highest probability of being involved in surface reactions (Pacella *et al*., 2010; 2012). As an independent confirmation, very good correlation was obtained plotting the production of HO° radicals against the content of Fe (II) at fibres surface (Fig. 2b). In order to check the correlation, data of fibrous tremolite samples from previous studies were added: results obtained strongly support the trend previously observed, revealing that Fe (II) at fibres surface actually controls the fibres surface reactivity, at least in terms of HO° radical production. Outstandingly, this is the experimental validation of the hypothesis formulated in (Fubini *et al.*, 2001; Gazzano *et al*., 2005; Favero-Longo *et al*., 2005) according to which iron exposed at the fibre surface is the main responsible for production of ROS. In addition, it must be noted that fibrous sample from Libby exhibited an anomalous behaviour with respect to the samples from Biancavilla (and tremolites) in terms of bulk Fe, surface Fe, chemical reactivity, and their relationships. In particular, in Fig. 2a it is visibly off trend, whereas in Fig. 2b it is aligned. This is the consequence of the very

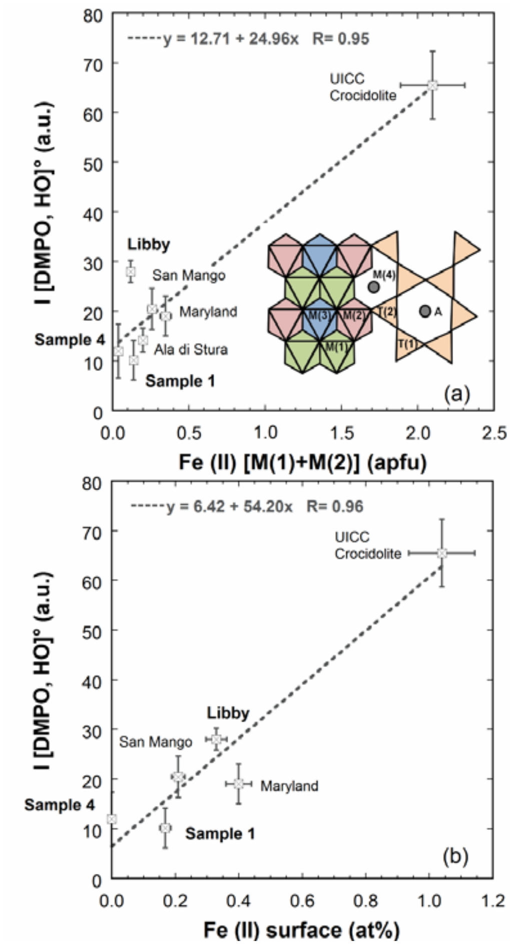

Fig. 2 - HO° radical production of the fibrous samples plotted against: a) their [M(1)+M(2)]Fe(II) content; b) their Fe(II) surface content from XPS data. The Fe(II) surface content of San Mango and Maryland tremolites and UICC crocidolite has been recalculated from Fantauzzi *et al*. (2010). (DMPO, HO)° data of San Mango, Ala di Stura and Maryland tremolites and of UICC crocidolite are taken from Pacella *et al*. (2010; 2012). The model of amphibole structure indicating cationic sites is adapted from Hawthorne *et al*. (2007).

limited surface oxidation previously reported, which justifies its relatively high content of surface Fe (II). The high production of HO° radicals here observed makes the Libby fibrous sample the most potentially harmful, even more than the tremolite asbestos. It is proved, in fact, that the production of HO° radicals is related to lipid peroxidation and promotes the production of malonaldehyde, which is recognized to be the most mutagenic and carcinogenic among the various products generated by the lipid peroxidation (Pacella *et al.*, 2012). Results presently obtained have been integrated with surface chemical analyses perfomed by X-ray photoelectron spectroscopy and a manuscript has submitted to the journal Analythical and Bionalythical Chemistry.

ACKNOWLEDGEMENTS

Many thanks to SIMP for financial support. M.E. Gunter generously provided the fibrous sample from Libby (Montana, USA). XPS results showed in this work were provided by Prof. A. Rossi and Dr. M. Fantauzzi of Dipartimento di Chimica Inorganica e Analitica of University of Cagliari (Italy).

REFERENCES

Andreozzi, G.B., Ballirano, P., Gianfagna, A., Mazziotti-Tagliani, S., Pacella, A. (2009): Structural and spectroscopic characterization of a suite of fibrous amphiboles with high environmental and health relevance from Biancavilla (Sicily, Italy). *Am. Mineral*., **94**, 1333-1340.

Cardile, V., Renis, M., Scifo, C., Lombardo, L., Gulino, R., Mancari, B., Panico, A.M. (2004): Behaviour of new asbestos amphibole fluoro-edenite in different lung cell systems. *Int. J. Biochem. Cell. Biol.,* **36**, 849-860.

Comba, P., Gianfagna, A., Paoletti, L. (2003): The pleural mesothelioma cases in Biancavilla are related to the new fluoro-edenite fibrous amphibole. *Int. Arch. Occup. Environ. Health*, **58**, 229-232.

Favero-Longo, S.E., Castelli, D., Salvadori, O., Belluso, E., Piervittori, R. (2005): Pedogenetic action of the lichens Lecidea atrobrunnea, Rhizocarpon geographicum gr. and Sporastatia testudinea on serpentinized ultramafic rocks in an alpine environment. *Int. Biodeter. Biodegr.*, **56**, 17-27.

Fubini, B. & Otero Aréan, C. (1999) Chemical aspects of the toxicity of inhaled mineral dusts. *Chem. Soc. Rev*., **28**, 373-381.

Fubini, B., Fenoglio, I., Elias, Z., Poirot, O. (2001): On the variability of the biological responses to silicas: effect of origin, crystallinity and state of the surface on the generation of reactive oxygen species and consequent morphological transformations in cells. *J. Environ. Pathol. Toxicol. Oncol.*, **20**, 87-100.

Di Paola, M., Mastrantonio, M., Carboni, M., Belli, S., Grignoli, M., Comba, P., Nesti, M. (1996): La mortalità per tumore maligno della pleura in Italia negli anni 1988-1992. *Rapporti ISTISAN,* **40**, 30 p.

Fantauzzi, M., Pacella, A., Atzei, D., Gianfagna, A., Andreozzi, G., Rossi, A. (2010): Combined use of X-ray photoelectron and Mössbauer spectroscopic techniques in the analytical characterization of iron oxidation state in amphibole asbestos. *Anal. Bioanal. Chem.*, **396**, 2889-2898.

Gazzano, E., Riganti, C., Tomatis, M., Turci, F., Bosia, A., Fubini, B., Ghigo, D. (2005): Potential toxicity of nonregulated asbestiform minerals: balangeroite from the western Alps. Part 3: depletion of antioxidant defenses. *J. Toxicol. Environ. Health*, **68**, 41-49.

Gianfagna, A., Ballirano, P., Bellatreccia, F., Bruni, B.M., Paoletti, L., Oberti, R. (2003): Characterization of amphibole fibers linked to mesothelioma in the area of Biancavilla, Eastern Sicily, Italy. *Mineral. Mag.*, **67**, 1221-1229.

Gunter, M.E., Brown, B.M., Bandli, B.R., Dyar, M.D. (2001): Amphibole asbestos, vermiculite mining and Libby, Montana: What's in a name? Eleventh Annual Goldschmidt Conference, Hot Springs, Virginia, U.S.A., abstract #3455.

Gunter, M.E., Dyar, M.D., Twamley, B., Foit, F.F., Jr., Cornelius, C. (2003): Composition, $Fe^{3+}/\Sigma Fe$, and crystal structure of non-asbestiform and asbestiform amphiboles from Libby, Montana, U.S.A. *Am. Mineral.*, **88**, 1970-1978.

Kamp, D.W. & Weitzman, S.A. (1999): The molecular basis of asbestos induced lung injury. *Thorax*, **54**, 638-652.

Hawthorne, F.C., Oberti, R., Della Ventura, G., Mottana, A. (eds.) (2007): Amphiboles: Crystal Chemistry, Occurence and Health Issues. *Rev. Mineral. Geochem*., **67**, 545 p.

Kane, A.B. (1996): Mechanisms of mineral fibre carcinogenesis. *IARC Sci. Publ*., **140**, 11-34.

Mazziotti-Tagliani, S., Andreozzi, G.B., Bruni, B.M., Gianfagna, A., Pacella, A., Paoletti, L. (2009): Chemical variability of a suite of fibrous amphiboles from Biancavilla (Sicily, Italy). *Per. Mineral.*, **78**, 65-75.

McDonald, J.C., McDonald, A.D., Armstrong, B., Sébastien, P. (1986): Cohort study of mortality of vermiculite miners exposed to tremolite. *Br. J. Ind. Med.*, **43**, 436-444.

Murray, R. (1990): Asbestos: A chronology of its origins and health effects: *Br. J. Ind. Med*., **47**, 361-365.

Oze, C. & Solt, K. (2010): Biodurability of chrysotile and tremolite asbestos in simulated lung and gastric fluids. *Am. Mineral.*, **95**, 825-831.

Pacella, A., Andreozzi, G.B., Fournier, J. (2010): Detailed crystal chemical study and iron topochemistry of asbestos in its natural setting: a first step to understanding its chemical reactivity. *Chem. Geol*., **277**, 197-206.

Pacella, A., Andreozzi, G.B., Fournier, J., Stievano, L., Giantomassi, F., Lucarini, G., Pugnaloni, A. (2012): Iron topochemistry, surface reactivity and *in vitro* toxicity of amphibole asbestos. *Anal. Bioanal. Chem.*, **402**, 871-881.

Paoletti, L., Batisti, D., Bruno, C., Di Paola, M., Gianfagna, A., Mastrantonio, M., Nesti, M., Comba, P. (2000): Unusually high incidence of malignant pleural mesothelioma in a town of the eastern Sicily: an epidemiological and environmental study. *Int. Arch. Occup. Environ. Health*, **55**, 392-398.

Robledo, R. & Mossman, R. (1999): Cellular and molecular mechanisms of asbestos-induced fibrosis. *J. Cell. Physiol*., **180**, 158-166.

Pugnaloni, A., Lucarini, G., Giantomassi, F., Lombardo, L., Capella, S., Belluso, E., Zizzi, A., Panico, A.M., Biagini, G., Cardile, V. (2007): In vitro study of biofunctional indicators after exposure to asbestos-like fluoro-edenite fibres. *Cell. Mol. Biol.*, **53**, 965-80.

Soffritti, M., Minardi, F., Bua, L., Degli Esposti, D., Belpoggi, F. (2004): First experimental evidence of peritoneal and pleural mesotheliomas induced by fluro-edenite fibres in Etnean volcanic material from Biancavilla (Sicily, Italy). *Eur. J. Oncol*., **9**, 169-175.

Van Oss, C.J., Naim, J.O., Costanzo, P.M., Gieise, R.F., Jr., Wu, W., Sorlung, A.F. (1999): Impact of different asbestos species and other mineral particles on pulmonary pathogenesis. *Clays Clay Miner*., **47**, 697-707.

Wilye, A. & Verkouteren, J. (2000): Amphibole asbestos from Libby, Montana: aspects of nomenclature. *Am. Mineral.*, **85**, 1540-1542.