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Physico-chemical properties of quartz from industrial manufacturing and its cytotoxic effects on alveolar macrophages: the case of green sand mould casting for iron production

Di Benedetto Francesco^{1,*}, *Elena Gazzano*^{2,3}, *Maura Tomatis*^{3,4}, *Francesco Turci*^{3,4}, *Pardi Luca A.*⁵, *Bronco Simona*⁵, *Fornaciai Gabriele*⁶, *Innocenti Massimo*⁶, *Montegrossi Giordano*⁷, *Muniz Miranda Maurizio*⁶, *Zoleo Alfonso*⁸, *Capacci Fabio*⁹, *Bice Fubini*^{3,4}, *Dario Ghigo*^{2,3} and *Romanelli Maurizio*¹

1 – Department of Earth Sciences, Università di Firenze, Firenze (Italy); 2 – Department of Oncology, Università degli studi di Torino, Torino (Italy); 3 - “G. Scansetti” Interdepartmental Center for Studies on Asbestos and other Toxic Particulates, Università degli studi di Torino, Torino (Italy); 4 – Department of Chemistry, Università degli studi di Torino, Torino (Italy); 5 – Institute for chemical and physical processes (CNR-IPCF), Pisa (Italy); 6 – Department of Chemistry, Università di Firenze, Sesto Fiorentino (Italy); 7 - Institute of geosciences and earth resources (CNR-IGG), Florence (Italy); 8 – Department of Chemical Sciences, Università di Padova, Padova (Italy); 9 – Health Agency of Florence, Firenze, Italy

KEYWORDS quartz, hole and Al centres, EPR/ESEEM, free radicals, cytotoxicity, ROS, NO, macrophages, health effects, carbon coating.

ABSTRACT

Industrial processing of materials containing quartz induces physico-chemical modifications that contribute to the variability of quartz hazard in different plants. Here, modifications affecting a quartz-rich sand during cast iron production, have been investigated.

Mineralogical composition, morphology, presence of radical species associated to quartz and reactivity in free radical generation were studied on a raw sand and on a powder recovered after mould dismantling. Cytotoxicity, ROS and NO generation elicited by the processed powder were evaluated on MH-S macrophages.

The following modifications were revealed: annealing of radiogenic defects; new radical species associated to a coverage by amorphous carbon ; contamination by iron oxides and increase of bioavailable iron; loss of surface reactivity in $\cdot\text{OH}$ generation and enhancement of $\cdot\text{CO}_2^-$ release; slight increase of the amount of respirable fraction. No cellular responses were observed with the processed powder.

These modifications show that some key physico-chemical properties of crystalline silica related to quartz toxicity are strongly affected by the green-sand mould casting process. .

Introduction

Professional exposure to quartz and cristobalite may cause relevant health effects, such as silicosis and lung out (**WHO-IARC, 2012**). Lung diseases, however, do not occur in all occupational settings since biological effects depend on physico-chemical properties of quartz, which may differ from one dust to another and may be modified during industrial processing (**Fubini, 1998; Donaldson and Borm, 1998; Donaldson et al., 2001**). Grinding, heating, mixing with other substances are the most common processes that can be experienced by silica-bearing materials and several efforts have been addressed in the past to better understand their effects on the quartz toxicity. Nevertheless, to date, few data on properties and biological activity of the workplace silica dusts are available. (**Fubini et al., 1995; Clouter et al., 2001; Cakmak et al., 2004; Fubini et al., 2004; Di Benedetto et al, 2014**).

Foundries operating the sand casting of the cast iron are listed among the industrial sectors with the longest tracks of exposure to silica, and larger occurrence of silica-related diseases (**Landrigan et al., 1996**). (**Erlich et al., 1988; Rees and Weiner, 1994; Gomes et al., 2001; Zhang et al., 2010**). Moreover, this industrial sector has a high carcinogenic risk: beyond silica, workers are also exposed to formaldehyde, metallic smokes, asbestos, and organic products related to the combustion process. Accordingly, the IARC (**WHO-IARC, 1987**) rated the production of cast iron and steel as having a proven carcinogenicity for humans. This rate was successively verified through epidemiological studies (**Sorahan and Cooke, 1989; Hansen 1997; Barbieri et al., 2005; Westberg et al., 2005; Hoshuyama et al., 2006**). The aim of this study is to investigate the main modifications of physico-chemical properties that may affect silica toxicity (**Fubini, 1998**), occurring to a quartz-rich sand in the “green sand” iron casting.

The quartz rich sands considered in the present study were analysed to assess the changes in the speciation of inorganic radicals associated with quartz and in the potential to generate free radicals in cell-free environment. Free surface radicals generated by reactive sites at the silica surface (particle-derived free radicals) together with oxidant species produced by phagocytes (cell-derived ROS) are thought to play a significant role, namely during a late stage of toxicity of silica (**Fubini and Hubbard, 2003; Castranova et al., 1996**). Inorganic radical speciation was carried out by Electron Paramagnetic Resonance (EPR) and Electron Spin Echo Envelope Modulation (ESEEM) spectroscopies. Free radical release was monitored by spin trapping technique associated to EPR spectroscopy. Changes in size and particle morphology, mineralogical composition, and amount of bioavailable iron were also evaluated.

The respirable fraction of the powder materials recovered after mould demolition was also tested for its ability in activating alveolar macrophages. Cytotoxicity and the potential to stimulate the production of Reactive Oxygen Species (ROS) and nitric oxide (NO) from cells were evaluated on a murine alveolar macrophage (MH-S) cell line and compared with cellular damage induced by Min-U-Sil, a fibrogenic quartz widely employed in experimental studies on silicosis and lung cancer.

Materials and methods

a) Sample description

The two samples belong to the processing line of the sand mould casting. In this process, cast iron coming from blast-furnace is introduced as raw material, melt and poured in moulds. These, in turn, are assembled by suitably modelling quartz (or quartz rich) sands. There are two ways to accomplish the mould assemblage by loose sands of mineral: pressing them (the so-called “*green*

sand” procedure) or mixing them with resins, and then pressing and forming them. In both cases, after casting, sands are recovered by the demolition of the mould and by homogenisation in a pan crusher. Then, they are fed back into the industrial cycle, to prepare a new mould. If mixed with resins, sands undergo a thermo-mechanical treatment, the “regeneration”, to remove most of the residual resin, before being re-used.

The two sands, hereafter labelled as I3 and I4, were sampled at a foundry operating just with the “green sand” processing line, so they were not mixed with resins. I3 consists of raw quartz rich sands, sampled before they enter in the industrial process. Sands are white at the sight (**Fig. S1**). I4 consists of powder materials sampled in the industrial process point where sands, recovered after the mould demolition, were stocked. The only relevant difference is the colour, turned to black (**Fig. S1**). Both samples were considered in previous studies by our group (**Romanelli et al., 2012; Di Benedetto et al., 2014**).

b) Mineralogical investigation

The samples were characterised as concerns the particle morphology and the phase composition through a set of techniques well established in the solid state investigations. Micromorphological information was provided by the Scanning Electron Microscopy (SEM), whereas phase composition was obtained through the combined use of X-ray Powder Diffraction (XRPD), Fourier Transform Infrared spectroscopy in the Attenuated Total Reflectance mode (FT-IR/ATR), and micro-Raman spectroscopy. Specific surface information was provided by the BET technique. Further experimental details are summarised in the **Supplementary Materials (Sec. A)**.

c) Speciation of inorganic radicals

The investigation of inorganic radicals was mainly performed through X-band EPR spectroscopy. In order to get a certain attribution of the radical species, three set of measurements were done: a preliminary survey through conventional continuous-wave (cw) EPR at room temperature, followed by a characterisation at 35 K, and by a set of measurements through pulsed EPR spectroscopy (field swept Echo-EPR and time-domain ESE experiments). The samples were analysed without any manipulation. Further details of the measurements are provided in the **Supplementary Materials (Sec. B)**.

d) Oxidative potential in cell free environment

Free radical generation ($\cdot\text{OH}$ and $\text{CO}_2^{\cdot-}$) was monitored by EPR spectroscopy using DMPO as trapping agent. Further technical details are given in the **Supplementary Materials (Sec. C)**.

e) Bio-available iron

The amount of removable ferrous and ferric irons on the sample surfaces was determined upon incubation of silica dusts in an aqueous solution containing ferrozine (a strong chelator specific for Fe^{2+} determination) or ferrozine and ascorbic acid, which fully reduces Fe^{3+} ions to Fe^{2+} , allowing the detection of total (Fe^{2+} and Fe^{3+}) removable iron. Details of the incubation procedure are provided in the **Supplementary Materials (Sec. D)**.

f) Cellular toxicity

Cytotoxicity was evaluated by measuring the release of LDH into the extracellular medium, and NO generation by quantifying the nitrite accumulation. DCFH-DA assay was used for the detection of intracellular ROS. All experiments were performed on murine alveolar macrophages (MH-S). Details are provided in the **Supplementary Materials**

Results

Mineralogical and chemical composition; granulometric distribution

In **Figure 2**, the secondary electron micrographs obtained on the I3 and I4 samples are shown. Sample granulometry is conceivably high. Particles exceed 300 μm , thus allowing the classification of the samples as “fine sands”, according to the Udden-Wentworth grain scale (**Blott and Pye, 2001**). Particles with a size considerably smaller are present only in I4 sample (**Fig. 2c**). This fine fraction is generated during the mulling step in the pan crusher. Both I3 and I4 showed a very low BET surface (0.9 and 1.1 m^2/g respectively), in agreement with their large average diameter.

The SEM investigation of the I3 particles reveals sub-rounded edges and corners, and relatively smooth surfaces. This suggests that almost no recent new surfaces were created by any industrial pre-treatment of the sands. Conversely, the particle surface of I4 sample is no longer smooth. The roughness of the surface can be attributed to the partial covering by additional phases.

XRD investigation of the I3 and I4 evidences the presence of quartz only (See Fig. X in Supplementary Materials). Therefore, we can conclude that impurities added to the sample during or after the cast are in an amorphous state, or it can be considered below the detection limit of XRD (under the adopted experimental conditions, 1 wt%).

The two vibrational spectroscopies were focused on the determination of the chemical nature of the coating. The ATR investigations (data not reported for brevity), while confirming the obvious presence of bands at $\sim 1000\text{ cm}^{-1}$ due to crystalline SiO_2 , rule out the possible presence in the coating of any other component but crystalline/amorphous carbon. These latter species are weak and badly determined in FTIR, due to small changes in the dipole moment. The microRaman spectra of the I3 and I4 samples, shown in the **Figure 3**, are more informative. The

I3 spectrum consists of some narrow bands (at 353, 400, 465, 695, 810 and 1159 cm^{-1}): all these bands can be safely attributed to modes arising from the SiO_2 crystal structure. In contrast, the spectrum of I4 reveals two marked features, a huge fluorescence background and the presence of two main bands at 1335 and 1580 cm^{-1} . These two bands can be attributed to the amorphous/graphitic carbon (whose reference band wavenumbers are 1360 and 1580 cm^{-1} , respectively). Namely, the first one is closely resembling that of the sp^3 carbon, the presence of which could thus be inferred, in admixture with some sp^2 carbon.

b) Speciation of inorganic radicals

The RT cw-EPR spectra of the I3 and I4 samples are shown in **Figure 4**. The spectrum of the I3 sample is characterized by the presence of three superimposed signals. The first line, definitely narrow (I in **Fig. 4a**), is very common in all the investigated raw quartz materials (e.g. **Romanelli et al., 2012**) and is attributed to inorganic radicals. The second one, a broad line (II in **Fig. 4a**), is attributed to some metal impurity. A obvious attribution is to Fe(III) oxides, which often exhibit signals in this region. From the asymmetric shape, and the width of the line (~ 85 mT), a tentative attribution to Oxygen coordinated Cu(II) is also proposed. This is in agreement with the trace element analyses reported by **Di Benedetto et al. (2014)**. The broad II line is probably tracing the contributions of both Fe(III) and Cu(II). The two lines, marked by arrows and labelled as III in **Figure 4a**, are due to Fe(III) in a strong ligand field, in a highly crystalline surrounding. We attribute them to Fe in quartz, in agreement with the literature.

The spectrum of the I4 sample is also characterized by the presence of two superimposed lines, both different from the formerly described. The main spectral line (IV in **Fig. 4b**) is a very broad (~ 106 mT in width), almost symmetric signal, whose features were already associated to Fe oxide crystalline or cryptocrystalline species (**Carbone et al., 2008**). In contrast, the V line (**Fig.**

4b) is very narrow and even more symmetric. The V line is attributed to radical species. Further details of the narrow lines of both samples are shown in **Figure 4c**. Line II presents a peak-to-peak width of ~ 1.6 mT and a more asymmetric shape than line V, whose peak-to-peak width is ~ 1.0 mT.

Cw-EPR spectral investigation was also performed at 35 K, in order to confirm the spectral attribution of the radical species. The line II of the spectrum of I3 at low temperature shows the characteristic superhyperfine ^{27}Al and ^1H structure of the h_{Al} centre, already described for this sample (Romanelli et al., 2012; Figure Y in Suppl. Mat.). No evidence of further spectral contributions was provided, thus pointing to the attribution of the radical speciation of I3 to the h_{Al} centres only (within the minimum detection limit). In contrast, the IV line of the spectrum of I4 does not reveal any hyperfine or superhyperfine structure at low temperature. The width of the signal, ~ 0.6 mT, is apparently reduced. The peak-to-noise ratio is ~ 1400 . On these bases, a final attribution of the IV line to an inorganic or organic radical cannot be fostered. Nevertheless, the attribution of this line to one of the known radical species associated to quartz is ruled out (Ikeya, 1993).

In the **Figure 5a**, the Echo-EPR spectra of the two samples are shown. The spectrum of I3 consists of an asymmetric broad absorption signal, where parallel and perpendicular g components are estimated at 337 ($g_{\parallel}=2.050$) and 344 mT ($g_{\text{perp}}=2.009$), respectively. The spectrum of I4 is characterized by the presence of a single narrow line, centred at 345.5 mT ($g\sim 2.00$), with a full width at half maximum of ~ 1.5 mT. The comparison of the EchoEPR spectra allows to better visualize the apparent difference in Zeeman anisotropy between the two species, as well as the difference in the position of their maxima.

To perform the 3-pulse time domain ESE spectra, the magnetic field value corresponding to the maximum intensity of each spectrum was applied. The results are shown in **Figure 5b**. Both patterns consist of two spectral features: the decay of the signal in the time domain and the nuclear modulation pattern. The I3 sample exhibits a faint nuclear modulation up to ≈ 400 ns; its intensity decreases in the remaining time domain and it is masked by the background noise. The overall decay of the signal looks like a monotonous exponential law. In contrast, the pattern of I4 sample exhibits a nuclear modulation that persists in the whole investigated time domain with an almost constant intensity. Moreover, the decay of the signal follows a monotonous exponential law as well, but it is by far more rapid than that of I3 sample.

The ESEEM of the two spectra, extracted from the time-domain data of **Figure 5b** by removing the decay of the 3P ESE, is shown in **Figure 6(a,b)** together with the corresponding Fourier transforms (**Fig. 6c, d**). The ESEEM of the I3 spectrum was already investigated in a previous study (**Romanelli et al., 2012**) and it is assigned to the species h_{Al} , thus confirming the result obtained by cw data. The modulation pattern (**Fig. 6a, c**) can be attributed to protons coupled to the Al paramagnetic centre (**Romanelli et al., 2012**). Conversely, the spectral features of the ESEEM of the I4 sample (**Fig. 6b**) cannot be related to the h_{Al} centre. The FT of I4 ESEEM (**Fig. 6d**) shows a strong peak at the frequency value corresponding to the Larmor frequency of ‘free’ protons, i.e. protons not chemically coupled to a paramagnetic centre, and located at distances where the dipolar coupling is almost negligible. A second feature is present at very low frequency values, corresponding to the large period of the experimental ESEEM of **Figure 6b**. The origin of this contribution has been found by simulation procedure (**Romanelli et al., 2012**); the best fit data (**Fig. 6e**) are obtained for a centre related via superhyperfine interaction to one surrounding ^{13}C nucleus at ~ 2.38 Å coupled by a Fermi constant $a_{iso} = 8.5$ MHz. Thus, we attribute the V line in Figure 4b to an inorganic carbonaceous radical species.

c) Bio-available iron

Bio-available iron, which corresponds to iron ions that can be easily removed from the surface of the particles, was detected in relevant amount on the dust recovered after mould demolition. The amount of iron released in solution upon 72 h was $7.4 \pm 0.8 \mu\text{mol}/\text{m}^2$ (average of two separate experiments) and less than half of that was in the reduced form. Conversely, raw sand released only trace of iron in the oxidized form.

d) Oxidative potential in cell-free conditions Free radical generation of the two sands is shown in **Figure 7**. Ability to catalyze: a) carboxyl radical ($\text{CO}_2^{\cdot-}$) release from the formate ion, used as “model” target molecule for homolytic cleavage of a carbon-hydrogen bond in endogenous molecules and b) hydroxyl radical ($\cdot\text{OH}$) release in the presence of H_2O_2 , mimicking contact of particles with the lysosomal fluid of alveolar macrophages, were investigated.

Both samples were able to generate carboxyl radicals (**Fig. 7A**) in the presence of ascorbic acid, a reducing agent present in the lung lining fluid, whereas no activity was found in a non-reducing environment (data not reported for brevity). The I4 sample was more reactive than the I3 sample, but less reactive than the reference quartz.

An opposite trend was observed for hydroxyl radical release. I3 generated $\cdot\text{OH}$ in a similar extent to Min-U-Sil quartz, whereas I4 was fully inactive (**Fig. 7B**).

e) Cellular tests

The fine fraction of the I4 sample was tested to evaluate the ability to induce a cytotoxic effect upon a 24 h incubation with murine alveolar macrophages. This fraction amounts to about 10% of the sample and contains 90% of particles with circle equivalent (CE) diameter $< 5 \mu\text{m}$ (the diameter of a circle with the same area of the particle). CE average diameter is $2.3 \pm 1.7 \mu\text{m}$, and modal value is $1.35 \mu\text{m}$.

Cellular damage was investigated only for sand recovered after mould demolition, because the raw sand does not contain relevant amount of fine particles (less than 1%). I4 was not cytotoxic and did not stimulate ROS or NO generation, at any of the concentrations tested, differently from Min-U-Sil quartz (**Fig. 8**).

Discussion

The quartz rich sand investigated in this study undergoes physical and chemical changes when used in the green sand casting. Sand size and morphology are only partially affected by the industrial process. At the beginning of the process, the sand consists of sub-rounded grains of quartz, with a mean dimension of $\sim 350 \mu\text{m}$. Size distribution is only slightly affected by a small increase in the content of fine particle (up to 10%). The morphology is preserved at the end of the production, the main difference consisting in a black carbonaceous coating. Covering of the quartz surface by the carbonaceous material is associated to a thermal shock undergone by the sands, which are kept in contact with a melt at 1150°C and abruptly cooled down to room temperature. The SiO_2 , under these conditions, reaches the transition temperature from the α -quartz to the β -quartz structures, transition which is fully reversible as associated to a displacive polymorphism. In contrast, tridimite is associated to quartz through an irreversible reconstructive polymorphism. The absence of tridimite in the XRD patterns confirms that the conditions of its formation were not reached.

The results of the vibrational spectroscopy measurements confirm that the coating consists mainly of carbon, prevailing in the sp^3 hybridization. The intensity of the bands arising from lattice vibrations of graphite indicates most of the carbon occurring in an amorphous state. The source of C, in the specific case of the green sand casting, has to be found in the melt Fe alloy,

which intrinsically contains C, and to which some more C is added to fulfil specific rheological requirements of the casting process. As a consequence, the abrupt lowering of the temperature after the cast causes the release of excess of both the intrinsic and added C towards the mould and it covers the sands in contact with the Fe alloy. This coating occurs at high temperature (T in the range 800-1200 °C). The role of the temperature has a drastic effect on the population of the radiogenic defects of quartz (**Ikeya, 1993**). In fact, they are not stable at high temperature, and annealing at 500 °C is able to annihilate the majority of h_{Al} radical centres in quartz. We were not able to detect the EPR and ESE traces of h_{Al} centres in the I4 sample. Taking into account the intrinsic detection limits of the ESE technique, and the partial interference of the carbonaceous radical (**Fig. 4a**), we can assess that, if present, the fraction of h_{Al} centres could not exceed few % units of the initial population (in I3). Both the coating by amorphous C and the change of radical speciation are supposed to locally occur in regions of the mould that are in contact with, or very near to, the cast. The fact that a random sample of sands, as I4, does not present detectable h_{Al} centres can be attributed to the re-cycling of the used sands in the production line (**Fig. 9**). The reuse of sands in subsequent passages of the same industrial process gives rise to a uniform exposure to C coating and to thermal shock.

Coating with carbonaceous is related to the addition of a new radical species to the sands. The occurrence of this addition and of the annihilation of the h_{Al} species points to a complete change of speciation of the radical population in the quartz sands. Indeed, most of the long living, and also the short living radicals proper of the crystalline silica, are warmed up to temperatures at which they undergo annihilation and/or recombination (**Ikeya, 1993**). In contrast, the carbonaceous radicals, added to the system, are stable even at the considered temperatures. With respect to this new radical, the quartz sands act only as a carrier. Carbon coating probably suppresses the potential of the raw sands to generate hydroxyl radicals in presence of H_2O_2 . This

effect was already observed in two standard quartzes that lost their reactivity after grinding with carbon soot (**Ghiazza et al., 2013**). Metal transition ions and quartz surface radicals may be responsible for $\cdot\text{OH}$ release. Hiding these surface species, either in a non-specific way (by extensive coverage with an inert phase) or through specific interaction (e.g. between quartz surface radicals and radicals present on carbon surface) could account for the reduction in reactivity towards H_2O_2 . Moreover, a scavenger activity of the carbon coating may also occur. Scavenging of $\cdot\text{OH}$ is, in fact, well documented in a number of carbon-based materials (**Fenoglio et al., 2006; Yin et al., 2009**). From this point of view, a reactivity played by the C coating also on some h_{Al} , residual from the thermal annihilation, cannot be excluded.

Note that both surface radicals and silica-generated radicals (**Fubini et al., 1995; Vallyathan et al., 1991; Castranova et al., 1996a**) play a role in the induction of oxidative reactions leading to macrophage membrane damage (**Dalal et al., 1990**), and contribute with cell-derived radicals (**Castranova et al., 1996b, Zang et al., 2000**) to the oxidative stress observed in lung following exposure to quartz particles (**Vallyathan et al., 1995, Holley et al., 1992; Janssen et al., 1992; Fubini & Hubbard, 2003**).

Nor I3 neither I4 were able to generate carboxyl radical in the absence of a reducing agent, but when ascorbic acid was added to the reaction medium, both samples were activated, in agreement with the higher reactivity of Fe(II) than Fe(III) in the cleavage of C-H bond (**Fenoglio et al., 2001; Tomatis et al., 2002**) Opposite to $\cdot\text{OH}$ release, $\text{CO}_2\cdot^-$ generation was slightly higher for I4 than for I3 sample. This is due to the presence of poorly coordinated ions (**Turci et al., 2011**), accessible for reaction (bio-available iron) because of unsatisfied coordination valences, associated to the iron rich phase. One has to recall that a net enrichment in iron (**Di Benedetto et al., 2014**) occurs during the processing (**Fig. 3B**). In this case, no scavenging effect towards carbon-centred radicals can be assumed.

Finally, despite the presence of quartz, I4 (fine fraction) was not able to induce a cytotoxic response or to stimulate ROS and NO generation in murine alveolar macrophages. The presence of the carbon coating, which modifies the silica surface, could account for the absence of cellular effects, as demonstrated for Min-U-Sil quartz loaded with carbon dust (**Ghiazza et al., 2013**) and already suggested by an old study evidencing loss of biological activity when quartz is ground with coal dust (**Le Bouffant et al., 1982**). Amorphous carbon per se shows low chemical reactivity, and does not induce cytotoxic effects (**Ma et al., 2007**) or inflammation responses (**Thomson et al., 1991**) in macrophage cells. However, a possible effect of the high temperatures experienced by the sand during casting process, which contribute to annihilation of quartz radical, may not be excluded.

Conclusions and perspectives

As expected, some key physico-chemical characteristics relevant to quartz toxicity are modified during the green sand mould casting for iron production. The raw quartz material has a negligible respirable fraction, and contains radical species (i.e. h_{Al}) ubiquitarily observed in quartz (**Ikeya , 1993**). The processed quartz is coated with amorphous carbon and contaminated by iron oxides, it has a moderate respirable fraction, and it shows an intriguing suppression of the original h_{Al} species accompanied by the occurrence of a new carbon-based radical. The presence of the carbon coating likely reduces the surface reactivity in terms of $\cdot OH$ generation with respect to the raw quartz, while contamination with the iron oxide may account for the slight increase in the generation of $\cdot CO_2^-$. Opposite to standard quartz, the respirable fraction of the processed quartz is not cytotoxic in murine alveolar macrophages. Interestingly, the absence of cytotoxic effect, the annealing of h_{Al} species, and the decreasing of the release of $\cdot OH$ species observed on the

processed quartz, is counterbalanced by the increasing of bio-available iron, by the appearance of C-radicals and the increment of generation of $\cdot\text{CO}_2^-$ species. Even if the surface reactivity of the processed sample is lower than that of Min-U-Sil standard quartz, our results do not allow to exclude a potential toxicity and indicate once again that only a multi-technique approach need to be followed to correctly describe the potential harm of a particulate.

Figure Captions

- Figure 2 Secondary electron micrographs of the I3 (a, b) and I4 (c,d) samples. The a) and c) micrographs were obtained at 50x (scale bar 200 μm), the b) and d) micrographs were obtained at 250x and 210x, respectively (scale bar 100 μm).
- Figure 3 Raman spectra of the A) I3 and B) I4 samples.
- Figure 4 Room temperature cw-EPR spectra of a) the I3 sample, b) the I4 sample, c) detailed view of the radical lines in a) and b) spectra. To be plotted to the same scale, the II line of I3 in c) is multiplied by a factor 30. Magnetic field values in milliTesla (mT), intensity in arbitrary units.
- Figure 5 a) field swept Echo-EPR spectra registered after a 2-pulse sequence on the I3 and I4 samples; b) time domain ESE decay pattern after a 3-pulse sequence on the I3 and I4 samples. Magnetic field values are expressed in Gauss. Time values are expressed in nanoseconds. Intensities are normalized.
- Figure 6 a) and b) ESEEMs and c) and d) Fourier transforms of the spectra of the I3 and I4 samples, respectively. e) Fourier transform of the ^{13}C simulated modulation. Time values are expressed in nanoseconds. Frequencies are expressed in MegaHertz. The plot in c) is modified after Romanelli et al. (2012).
- Figure 7 Free radical generation from aqueous suspension of the raw sample and of the sample recovered after mould demolition. EPR spectra of $[\text{DMPO}-\text{CO}_2^-]\cdot$ ($a_{\text{N}} = 15.6$ G and $a_{\text{H}}=19$ G) (A) and $[\text{DMPO}-\text{HO}]\cdot$ ($a_{\text{N}} = a_{\text{H}}=14.9$ G) (B) adducts of (a) raw sand (I3), (b) sand recovered after mould demolition (I4), and (c) reference quartz (Min-U-Sil). Hydroxyl radicals were obtained in the presence of H_2O_2 , while carboxyl

radicals via homolytic cleavage of a C-H bond in the formate ions. All spectra have been collected after 60 min of incubation of the dusts with target molecules.

Figure 8 Effect of I4 fine fraction on MH-S murine alveolar macrophage cells. MH-S cells were incubated for 24 h in the absence or presence of 20, 40, 80, 100 $\mu\text{g}/\text{cm}^2$ of I4 fine fraction \square (\bullet) Min-U-Sil quartz (\blacksquare), used as reference quartz. A) Cytotoxicity measured as LDH leakage following cell membrane damage, B) ROS (reactive oxygen species) production and C) NO (nitric oxide) production, measured as nitrite concentration. Data are presented as means \pm SE (n = 3). Vs CTRL: * p < 0.05, ** p < 0.01

Figure 9 Schematic representation of the cycle experienced by the quartz (qz) sands in the green sand casting process.

Corresponding Author

* corresponding author: Francesco Di Benedetto - Department of Earth Sciences, Università di Firenze, Firenze (Italy); francesco.dibenedetto@unifi.it

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally.

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