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Ageing of dissolved copper and copper-based nanoparticles in five different soils: short term kinetics vs long term fate

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Abbreviations: Cu-NMs, copper-based nanomaterials; CuO-NPs, copper (II) oxide nanoparticles; CuS-NPs, copper (II) sulfide nanoparticles; Fh, ferrihydrite; Gt, goethite; HA, humic acid; LCF, linear combination fitting; XANES, X-ray absorption near edge structure; NOM, natural organic matter

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CORE IDEAS

- Short term reactions of Cu are dependent on the Cu form (dissolved vs NP) and soil chemistry

- In low pH soils, CuO-NPs dissolved rapidly and behave similarly to dissolved Cu

- CuO-NPs persisted longer in alkaline soils and in soils with high NOM content

- In the long term, Cu, CuO-NPs and CuS-NPs transform into Cu bound to FeO(OH) or NOM

- The long term fates are largely independent of the original form

ABSTRACT

With the growing availability and use of copper based nanomaterials (Cu-NMs), there is increasing concern regarding their release and potential impact on the environment. In this study, the short term (≤ 5 days) ageing profile and the long term (4 months) speciation of dissolved Cu, copper oxide (CuO-) and copper sulfide nanoparticles (CuS-NPs) were investigated in five different soils using X-ray absorption spectroscopy (XAS). Soil pH was found to strongly influence the short term chemistry of the Cu-NMs added at 100 mg/kg above background. Low pH soils promoted rapid dissolution of CuO-NPs that effectively aligned their behaviour to that of dissolved Cu within 3 days. In higher pH soils, CuO-NPs persisted longer due to slower dissolution in the soil and resulted in contrasting short term speciation compared to dissolved Cu, which formed copper hydroxides and carbonates that were reflective of the soil chemistry. Organic matter appeared to slow the dissolution process but in the long term, the speciation of Cu added as dissolved Cu, CuO-NPs and CuS-NPs were found to be same for each soil. The results imply that in the short term Cu-NMs may exhibit unique behaviour in alkaline soils compared to their conventional forms (e.g. in the event of an adverse leaching event), but in the long term (≥ 4 months), their fates are dictated by the soil properties and are independent of the initial Cu form, and are likely to present minimal risk of nano-specific Cu-NM impact in the soil environment for the concentration studied here.

INTRODUCTION

The advent of nanotechnology has introduced novel products containing engineered

nanomaterials (ENMs) to many industries around the world. They include metallic

nanoparticles such as silver or gold, metal oxide nanomaterials (e.g. zinc or titanium oxides), as

well as composite or polymeric nanomaterials (Forbrugerrådet Tænk, 2013; Woodrow Wilson

Institute, 2005, rev. 2013). The increasing abundance and availability of ENMs has led to

concerns regarding their behaviour in the environment, largely because their unique, specific

properties have created uncertainties about their likely environmental fate and effects. Many

studies have now focussed on comparing the environmental chemistry and ecotoxicology of

metal ions against their nanoparticulate counterparts, but the vast majority of studies to date

have focussed on silver and zinc. Far less is known about copper-based nanomaterials (Cu-

NMs), yet these are used increasingly widely, particularly as antifungal/antifouling agents

(Anyaogu et al., 2008), and also in electronics, ceramics, health and personal care products

(PCP) (Forbrugerrådet Tænk, 2013).

Soil is one of the critical environmental endpoints in the environmental risk assessment

of copper. Copper, in its variety of forms, has been used in agriculture for a long time.

Bordeaux mixture (copper hydroxide and calcium sulfate) has been used as an agricultural

fungicide since the 1800s (Flores-Vélez et al., 1996), and chromated copper arsenate (CCA) has

long been used to treat timber as it provides highly effective protection against termites and

fungi (Hingston et al., 2001). While CCA itself is no longer widely used due to its potential to

leach toxic arsenic and chromium (Hingston et al., 2001), other copper based materials, and

increasingly Cu-NMs, (Civardi et al., 2015; Evans et al., 2008), are still used to treat timber. They

also continue to be used as active ingredients in agricultural fungicides (e.g. Kocide®) as copper-based fungicides are accepted for use on organically certified products in many countries (Bruggen and Finckh, 2016). Copper from these sources could potentially be released into the environment in its native form (e.g. CuO or Cu(OH)$_2$) or through leaching of more soluble or mobile forms (e.g. Cu(OH)$^+$, organically bound Cu). In other scenarios, Cu-NMs from PCPs and supplements (e.g. MesoCopper®, Ionic Colloidal Copper) may enter the sewerage system (as other ENMs from consumer products have been shown to) where they are likely to partition into the solid phase throughout the wastewater treatment process and hence become a component of the sewage sludge/biosolids. In these scenarios it is likely that they would exist in the biosolids as a mixture of copper sulfides (Cu$_x$S$_y$) and humic acid bound Cu (Donner et al., 2013; Donner et al., 2011). Since copper can exert toxic effects on microbial communities (Frenk et al., 2013; Li et al., 2015; Moore et al., 2016), nematodes (e.g. Amorim and Scott-Fordsmand, 2012; Unrine et al., 2010), and plants (Anjum et al., 2015), that may depend on both form and speciation, understanding the fate of Cu-NMs as influenced both by the initial form and subsequent transformation is of key importance in understanding their ecological impact.

Copper is an essential micronutrient for plants and understanding Cu-NM chemistry in soil environments could thus play a crucial role in optimising their beneficial use and appropriate application as fertilisers. However, Cu is also a known contaminant, and due to its reactive, redox-active nature, various soil properties may affect its behaviour over time. For example, pH has a strong influence on Cu soil chemistry and was found to have a significant impact on its speciation and lability (Ma et al., 2006a; Ma et al., 2006b). In these studies,

precipitation as $Cu(OH)_2$ or malachite ($Cu_2CO_3(OH)_2$) was suggested to be important in high pH soils (e.g. calcarosols), and Mamindy-Pajany et al. (2014) recently identified malachite by X-ray absorption near-edge structure (XANES) spectroscopy in a biosolid-amended calcarosol. Liu and Wang (2004) found that $CuCO_3$, CuO and Cu_2O were also identified using XANES analysis. Another important factor in the regulation of soil Cu chemistry, particularly in the long term, is soil organic matter. Copper has been found to associate strongly with SOM across a wide range of soils (Strawn and Baker, 2008; Strawn and Baker, 2009), and the soluble fraction of Cu is almost exclusively complexed to organic ligands (Sauvé et al., 1997). Adsorption to inorganic mineral surfaces (e.g. iron oxyhydroxide, calcium carbonate) is also known to occur but to a lesser extent, and this process depends on both the pH (McBride et al., 1998) and the ratio of the inorganic to organic adsorbent (Alcacio et al., 2001). Through these studies and others, conventional Cu speciation in soils has been studied extensively, however, very few studies have focused on Cu-NM behaviour and speciation in soils (Collins et al., 2012; Gao et al., 2017; Julich and Gäth, 2014; Shah et al., 2016).

In this study, we have examined the behaviour of Cu applied to soils as dissolved copper ($Cu(NO_3)_2$), copper oxide (CuO-) and copper sulfide nanoparticles (CuS-NPs), using XANES spectroscopy to determine their reaction profiles and eventual speciation in five soils of varying properties. Both the soil pH and the form of Cu was found to influence the short term reactions in soil: dissolved Cu partitioned very quickly into major compartments, while pH-dependent dissolution of CuO-NPs controlled the behaviour of Cu applied in this form. However, in the long term (4 months) Cu speciation was the same regardless of the initial form for each soil, with the major differences arising from soil pH and organic carbon content.

MATERIALS AND METHODS

Synthesis and characterisation of copper oxide and copper sulfide nanoparticles

Copper oxide nanoparticles (CuO-NPs) were synthesised from copper chloride

(CuCl$_2$.2H$_2$O) according to a wet chemistry method described by Misra et al. (2011). Briefly,

0.02 M CuCl$_2$.2H$_2$O was dissolved in 150 mL of ultrapure water. The solution was then heated

to 100 °C followed by rapid addition of 0.6 g NaOH. This resulted in the formation of a black

precipitate, which was centrifuged out and repeatedly washed several times with MilliQ water

to obtain pure-phase CuO-NPs. The synthesised nanoparticles were characterised by dynamic

light scattering (DLS; Nicomp 380 ZLS, Particle Sizing Systems) and scanning electron microscopy

(SEM; Quanta 450 FEG-ESEM, FEI Company). The SEM images show that the CuO-NPs took the

form of elongated, non-spherical particles (Figure 1) and that the indicative hydrodynamic

diameter (D_h) measured by DLS was 47.8 ± 6.2 nm.

Copper sulfide nanoparticles (CuS-NPs) were synthesized through the direct sulfidation

of CuO NPs described by Moore et al. (2016). In short, 1 gram of *ca.* 50 nm CuO-NPs (Sigma

Aldrich) were mixed with 2.5 g of gum arabic (Sigma Aldrich) in 900 mL of DI water and

dispersed using a sonicating probe (Branson Model 250). To this suspension, 100 mL of of 0.1M

Na$_2$S (Sigma Aldrich) was added and allowed to react for one week under constant stirring. To

remove excess gum arabic and Na$_2$S, the suspensions were centrifuged and resuspended in DI

three times. The CuS-NPs were characterized previously in filtered porewater by DLS (Malvern

Zetasizer Nano) and transmission electron microscopy (TEM, FEI Tecnai G2 Twin). These

particles were characterized previously: the CuS-NPs are aggregates of non-spherical particles (primary particle size of 12.4 ± 4.1 nm) and DLS confirmed that these particles exist as aggregates (185 ± 11 nm) in an aqueous suspension (Moore et al., 2016).

Five different soils were selected from Australian sites (South Australia: Lenswood, Barren Ground, Minnipa and Pimpinio; Victoria: Hamilton) to cover a range of soil parameters including pH (5 – 8), total organic carbon (TOC; 1.3 – 5.5 %), water holding capacity (WHC, 26.5 – 43.7 % w/w) and background copper concentration (1 – 9.6 mg/kg) (Table 1). Five grams of each soil were brought to 30 % WHC for each treatment prior to adding Cu. Dissolved copper, CuO- or CuS-NPs were added to give final Cu concentrations in each soil of 100 mg/kg above background (Table 1) and WHC was increased to 60%. The samples were aged at 18 °C and 60% WHC in the dark for 4 months, and subsampled for analysis at 3 days, 5 days and 4 months. The samples were aerated by once daily mixing during the first part of the experiment (i.e. up until the 5 day sampling point), and by weekly mixing thereafter up until 4 months. The 0 day samples were prepared on site at the synchrotron immediately before spectral acquisition (t< 1h).

Synchrotron X-ray Absorption Spectroscopy

X-ray absorption spectra were collected using the wiggler XAS Beamline at the Australian Synchrotron. The storage ring was operating at 3 GeV and 200 mA in top-up mode. Spectra were recorded at the Cu-K absorption edge (8979 eV) using a liquid N_2 cooled Si (111) double crystal monochromator. The sample spectra were collected in fluorescence using a 100-

element HP-Ge detector (*Canberra)*, in duplicate, and Cu foil reference spectra were recorded simultaneously in transmission during sample analysis. The reference spectra were used for energy calibration and alignment purposes (1st derivative inflection point at 8979 eV, absorption peaks at 8981.5, 8993.5 and 9025 eV).

Inorganic Cu standards analysed included copper (I) –oxide (Cu_2O), -sulfide (Cu_2S), copper (II) -nitrate ($Cu(NO_3)_2$), -chloride ($CuCl_2$), -sulfide (CuS, covellite), -sulfate ($CuSO_4$), -oxide (CuO), -carbonate ($CuCO_3$), and -phosphate ($Cu_3(PO_4)_2$), as well as Cu bound to organic ligands: cysteine, histidine, aspartate and arginine. Copper sorbed to iron oxyhydroxides (ferrihydrite, Fh, and goethite, Gt) were used to model Cu sorbed on mineral surfaces. Native copper in Suwannee river natural organic matter (SR-NOM) was used as a model for copper bound to NOM, as well as SR-NOM that was allowed to dialyse (MWCO = 13.5 kDa) for 24 h against 100 mM $Cu(NO_3)_2$. The CuO-NPs standard spectrum was acquired from a paste made by mixing the suspension with cellulose powder. The spectra after 4 months were collected at the Materials Research Collaborative Access Team Beamline BM10, Sector 10, at the Advanced Photon Source (APS) of the Argonne National Laboratory (Kropf et al., 2010).

Inorganic compounds were used as purchased from commercial sources (Sigma Aldrich, NSW, Australia; Thermo Fisher Scientific, VIC, Australia), except for two minerals: CuS from the South Australian Museum which was freshly cleaved from its mineral (covellite) prior to spectral measurement, and malachite from the Smithsonian Natural History Museum. The organic Cu compounds were synthesised by reacting $Cu(NO_3)_2$ with each ligand and freeze-drying. Each material compound was diluted to ca. 200 mg/kg in cellulose powder for XANES

acquisition. All standard spectra were acquired on the Australian Synchrotron except Cu-HA,

Cu-goethite and malachite spectra, which were acquired at the APS.

The fluorescence XAS spectra were averaged using the pre-processing tool Sakura.

Athena (Ravel and Newville, 2005) was used for background subtraction and normalisation over

the range of -120 eV to +230 eV around the Cu K-edge. Principal Component Analysis and

Target Transformation was performed was performed using the SixPack program (See

Supplementary Information file for details). Linear Combination Fitting (LCF) over the XANES

region (-15 eV to +100 eV) was performed with Athena, using a combination of up to 4

standards out of 15 standards selected for analysis, 8 of which were selected on the basis of

low SPOIL values (<3.0) by Target Transformation (TT, see Supplemental Table S2), and up to 7

additional standards selected as likely to be relevant on the basis of related experiments and

literature.

RESULTS AND DISCUSSION

Short term chemistry of dissolved Cu and CuO-NPs in soils

The Cu K-edge XANES spectra of the soils during the first 5 days after Cu addition

indicated significant variation in Cu behaviour and speciation in these different soils (Figure 2).

Linear Combination Fitting analysis (Table 2) revealed that the short term reaction profiles of

dissolved Cu and CuO-NPs were dependent on the soil properties (Table 1) and in particular,

there was a strong dependence on the soil pH for both species. In low pH soils, CuO-NPs

dissolved faster and behaved similarly to $Cu(NO_3)_2$ within the 5 days, while in more alkaline soils

CuO-NPs reacted more slowly and persisted longer in the soil. This is particularly evident in the

Lenswood soil, which was highly reactive towards CuO-NPs. Although characteristic CuO-NPs

XANES spectra are evident in all other soils at Day 0, in the Lenswood soil, the CuO structure

was immediately lost. By Day 3, the XANES profiles from the soils spiked with dissolved Cu and

CuO-NPs were almost identical in appearance, and they did not change significantly by Day 5.

This indicates that the fast reactions were largely completed by Day 3. The LCF results support

this observation, revealing near identical speciation (within the error of the LCF procedure) for

both dissolved Cu and CuO-NPs at Days 3 and 5, consisting predominantly of Fh and NOM-

bound Cu with very low residuals (R factor $\leq 1.2\times10^{-4}$).

In comparison, alkaline soils (Pimpinio and Minnipa, both pH 8.0) reacted more slowly

with CuO-NPs, with Minnipa retaining nearly half of the initial structure even after 5 days of

incubation according to LCF decomposition. The slowly released Cu is then redistributed to the

iron oxyhydroxide and NOM phases that were also observed in the low pH soils. Thus, while

the rate of the CuO dissolution is different, the partitioning of Cu from CuO-NPs tend towards a

similar fate in both low and high pH soils.

Evidently, pH alone cannot explain all the differences. For example, Barren Ground soil

has a lower pH than Lenswood but the reactivity towards CuO-NPs are apparently suppressed

in comparison; a clear CuO component is evident in the Barren Grounds Day 0 spectrum.   This

may be due to the higher soil organic matter (SOM, measured by total organic carbon, TOC)

that may be providing some protection for CuO against dissolution by partially passivating the NP surface (Conway et al., 2015) or by inducing aggregation (Sousa and Teixeira, 2013). Nevertheless, by Day 3 CuO is no longer identified in the LCF and it is clear that CuO-NPs behaviour in the short term are largely determined by the soil pH.

A slightly different observation is made for Cu introduced into soils as dissolved $Cu(NO_3)_2$. In low pH soils, the partitioning process is very fast and the Cu is rapidly distributed into similar Cu phases as those found in the CuO-NPs spiked soils. One notable difference is observed with Lenswood soil at Day 0, where substantially higher Cu_2S formation is indicated by the LCF analysis. It is not clear why this has formed and given the comparatively poor fit for this spectrum (R factor = 7.0×10^{-4}), it is possible that the LCF analysis is not accurately predicting the composition for this sample. Where it appears in all other spectra, the Cu_2S contribution is relatively small and may be forming within anaerobic micro-environments in the soil (e.g. micro-cavities) but it cannot be determined from this study. Additional studies including more standards and EXAFS analysis may provide some further clues.

In contrast to the low pH soils, $Cu(NO_3)_2$ added to high pH soils tends to immediately form $Cu(OH)_2$ or $CuCO_3$, both of which are likely in alkaline environments, but are not observed in the CuO-NPs spiked soils. This can be rationalised based on solubility of the species Cu involved. When Cu is introduced as dissolved Cu, all of the added Cu is theoretically available to react with dominant moieties or surfaces. If all Cu was able to instantly dilute throughout the entire moisture content of the soil, Cu concentration would be ca. 6.5 mM (100 mg/kg = 1.57 mmol/kg, 1 kg soil has added 60% of soil WHC, which is ca. 40% for alkaline soils, so 1.57

(mmol/kg) ÷ (0.6×0.4) (L/kg) = 6.5 mmol/L). At pH > ca. 6, this concentration would result in

$Cu(OH)_2$ precipitation (Albrecht et al., 2011).   In contrast, when Cu is added as CuO-NPs, since

the Cu release is slow the dissolved Cu concentration will not exceed Ksp and does not

precipitate to $Cu(OH)_2$, thus it is likely to sorb to FeO(OH) or NOM.   It is also worth noting that

although malachite ($Cu_2(CO_3)(OH)_2$) can often be observed in calcarosols (Mamindy-Pajany et

al., 2014; McBride and Bouldin, 1984), it nor the related $CuCO_3$ was not observed in the

calcarosol included in this study (Minnipa). However, the addition of free Cu to the Pimpinio

soil, which has a higher EC (that may contain high dissolved carbonate), readily brought about

$CuCO_3$ formation. Thus, the short term speciation of Cu added as $Cu(NO_3)_2$ will more likely

reflect the chemistry of the soil solution than will the short-term transformation products of Cu

added as CuO-NPs.

Copper speciation after 4 months

While the short term kinetics and speciation were highly dependent on the initial form

of the Cu applied and soil properties, the long term fate of Cu in soil appears to be similar and

therefore largely independent of the species of copper introduced. As shown in Figure 3, the

XANES spectra from $Cu(NO_3)_2$, CuO-NPs and CuS-NPs aged in the soils for 4 months are near

identical to each other. It is worth noting that despite their low K_{sp} ($6×10^{-37}$), CuS-NPs are

susceptible to oxidative dissolution under aerobic conditions and are reportedly more soluble

than CuO-NPs (Ma et al., 2014). It has also been observed that while Cu was found as various

sulfides in freshly prepared biosolids, a significant proportion had transformed to HA or iron

oxyhydroxide-bound Cu after stockpiling (Donner et al., 2011). Therefore, it is reasonable that

CuS-NPs were not identified in the LCF (Supplemental Table S3). This contrasts with Ag_2S-NPs, whose low K_{sp} and kinetic stability has a direct consequence on in its persistence in soil as Ag_2S (Pradas Del Real et al., 2016; Sekine et al., 2015; Wang et al., 2016).

In general, the spectral profile for Cu in soils after 4 months is very similar to Cu bound to iron oxyhydroxides – both Cu-Gt and Cu-Fh could contribute to the final speciation according to LCF, with a smaller but consistent contribution from Cu bound to organic matter (NOM, HA). The predominance of Cu bound to iron oxyhydroxide is, however, in some disagreement with previous reports (for example (Nielsen et al., 2015; Strawn and Baker, 2009)), where SOM was found to be the dominant Cu partition. On the other hand, many of these studies focused on soils with pH < 7 that contained comparatively high SOM (as measured by TOC), so it could be that Cu bound to SOM had a stronger representation.

At the same time, the proportions predicted by LCF in this study may not provide the full picture. For example, a qualitative comparison of the Pimpinio spectra (pH 8.0) suggests a second "peak" is present and more distinctly visible in alkaline soils with an inflection point at 9008.5 eV. The latter inflection appears to be a common feature amongst our standards that have Cu(II) associated with an inorganic oxygen species as can be seen in Cu-Gt, -Fh, $Cu(OH)_2$, $CuCO_3$ for example (Figure 4). This may therefore indicate that there is more Cu bound to FeO(OH) in the alkaline soils contrary to the LCF results. This is plausible given that: i) Fh or Gt have a point of zero charge (PZC) around 6 to 9 (Schwertmann and Fechter, 1982), the binding of Cu (II) to FeO(OH) is expected to be more significant at higher pH; and ii) the ratio of Cu bound to FeO(OH) (Gt) versus NOM (HA) would increase with pH as reported by McBride et al.

(1998). This feature is also present, although to a lesser extent, in the aged Minnipa soil but is not visibly apparent in the other soils where pH < 7. Possible explanations for the discrepancy between the visual vs LCF interpretation may be due to additional complexes formed at the FeO(OH)-NOM interface (e.g. "Type-A complex" similar to observations reported by Alcacio et al. (2001)), or the fact that the 4 month sample spectra were acquired on a different synchrotron beamline.

Finally, it is important to note that Cu bound to FeO(OH) used here are still models for Cu bound to an iron-containing mineral species present in the soil. For a more detailed investigation, a microfocus beam is necessary so that elements and species can be co-localised to provide spatially resolved information, which may lead to further refinement and discrimination of individual Cu components (Proffit et al., 2015).

Further discussions

The ageing process of Cu (as $CuCl_2$) in soils have been investigated and successfully modelled previously via a combination of three main mechanisms: 1) precipitation of Cu on soil mineral surfaces, 2) occlusion within soil organic matter and 3) diffusion into the micropores of clays and other minerals (Ma et al., 2006a; Ma et al., 2006b). Soil pH was reported to be the key factor determining the lability of Cu in the soils covering a range of soil parameters, with lower lability observed at higher pH due to precipitation of $Cu(OH)_2$ or malachite. Given the time scale in our study (up to 4 months), mechanisms 1 and 2 would be the main contributors to the ageing process, with a smaller contribution from diffusion based mechanism, 3. In

addition, since the short term Cu speciation (up to 5 days) by LCF suggested that the ageing pathway were dependent on the initial form (i.e. dissolved or Cu-NMs), an extra term would be required to account for the form in which the Cu is introduced. Furthermore, this term would need to decay over time since the Cu speciation has converged to the same fate regardless of the initial form in the long term (4 months). While the development of a mathematical model to account for these additional, potentially complex "convergence terms" is beyond the scope of this study, it is likely that this additional term would have a dissolution-based component, in agreement with the recent study by Gao et al. (2017).

Clearly, this convergence term would depend on a range of soil parameters such as pH and organic matter content as evidenced by the short term reaction profile. In low pH soils, this would be negligible since CuO-NPs entering these soils quickly dissolve and behave as dissolved Cu. Thus, if Cu lability is taken as a direct indicator of adverse soil ecological impact, then CuO-NPs in these soils would largely present the same hazard as Cu introduced as dissolved Cu. On the other hand, in alkaline soils, where CuO-NPs persisted longer, the dissolution process would have a greater influence on the overall attenuation rate. Furthermore, it must be recognised that the dissolution process is also a function of the NPs properties such as the size, shape, surface coating and aggregation state, as well as external factors such as the temperature. It may also be affected by the NP concentration, especially for poorly soluble NPs if the porewater becomes saturated with respect to the solid NP phase. If occlusion of Cu within organic matter in its NP form (i.e. prior to dissolution) also occurs, that will slow the convergence time. All these factors would inevitably add significant complexity to a comprehensive attenuation model, but a survey of CuO/CuS-NPs dissolution rates across a

range of soils may provide a semi-empirical term to extend the existing model to account for this process.

If Cu-NMs are to present any nano-specific risks, the results of this study imply that it would be greater in alkaline soils where CuO-NPs persisted for a longer time. For example, it has been reported that CuO-NPs can behave differently within soils not only in their speciation but also in their adsorption behaviour (Julich and Gäth, 2014) and in their effects on soil (micro-)organisms (e.g. Buffet et al., 2013; Unrine et al., 2010). These differences may be an important consideration in assessing their ecological risk under acute exposure scenarios in alkaline soils. Another possible scenario where such differences may manifest is where Cu-NMs are applied to soils in a matrix rich in organic matter such as biosolids (if Cu exist in nano form in biosolids) since it may be a contributor to their persistence in soil. In low pH soils however, they are unlikely to be significant unless under a considerably high exposure scenario in an organic matter rich soil. If the exposure occurs after the Cu-NMs have had time to age, the differences are likely to be minimal.

The results of this study also have implications for understanding the efficacy of nano-enabled fertilizers of fungicides. If the nano-specific properties of Cu-NMs are to be exploited in such applications, their potential benefits are likely to be realised in alkaline soils or SOM-rich soils soon after application. In soils that do not meet this criteria, other amendments to raise the pH (e.g. liming) or its organic matter content (e.g. biosolid application) may increase the effectiveness of the applied Cu-NMs. However, such strategies will need to be examined

carefully so that the potential environmental and ecological drawbacks do not outweigh the benefits, if any, gained from any amendments or Cu-NMs applications.

CONCLUSIONS

We have investigated the short term ageing profile and long term fate of dissolved Cu and Cu-NMs in five soils with different properties using Cu K-edge XANES spectroscopy. The short term reactions over the first 5 days of incubation were affected by both the initial form of Cu added and the soil characteristics. In particular, the soil pH had a significant impact on the behaviour of Cu in these early stages, with the low pH soils facilitating rapid dissolution of Cu-NMs that effectively aligned their behaviour to that of dissolved Cu. Soil organic matter is also likely to slow this process. At higher pH, Cu added as dissolved Cu immediately precipitated to form $Cu(OH)_2$ and/or $CuCO_3$ while CuO-NPs dissolved slowly and were partitioned directly into Cu bound to $FeO(OH)$ or NOM. In the long term (4 months), the XANES spectra indicated that all Cu were a combination of inorganically bound Cu to $FeO(OH)$ and organically bound copper to NOM with slightly different, pH-dependent proportions, with negligible contributions from other inorganic precipitates. Only minor differences were present in the spectra across the different Cu species in a given soil. These results imply that in the short term Cu-NMs may exhibit unique risks in alkaline soils compared to their conventional forms (e.g. in the event of an adverse leaching event), and there is a need to consider the effect of various factors relating to the Cu-NMs (e.g. concentration), the soil (e.g. SOM content) and external conditions (e.g. temperature). However, in the long term, it is likely that their fates are dictated by the soil

properties, independent of the initial Cu form, and are thus likely to present minimal risk of nano-specific Cu-NP impact in the soil environment.

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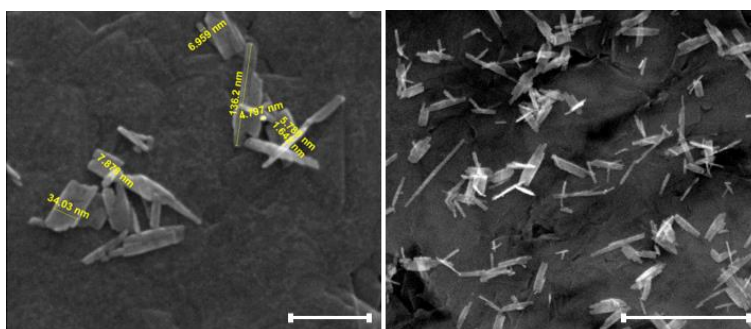
Figure 1. SEM images of the synthesized CuO-NPs showing the nanoscale needle like structures. Scale bars for the left and right images indicate 100 and 400 nm respectively.
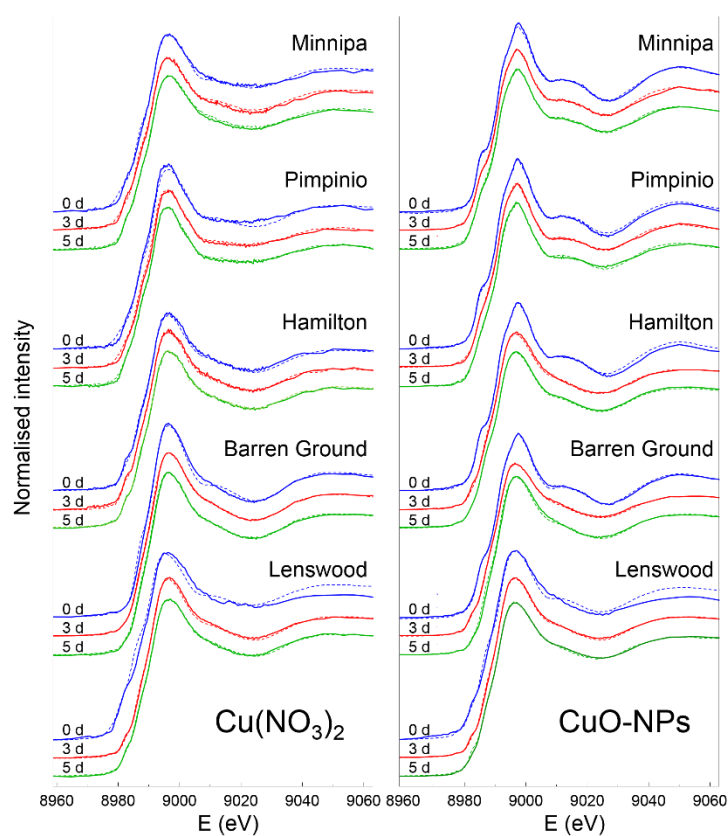

Figure 2. Cu K-edge XANES spectra of Cu(NO₃)₂ (left) and CuO-NPs (right) after incubation in soil for 0 days (blue), 3 days (red) and 5 days (green). The LCF results are shown as dotted lines overlayed on each spectrum.
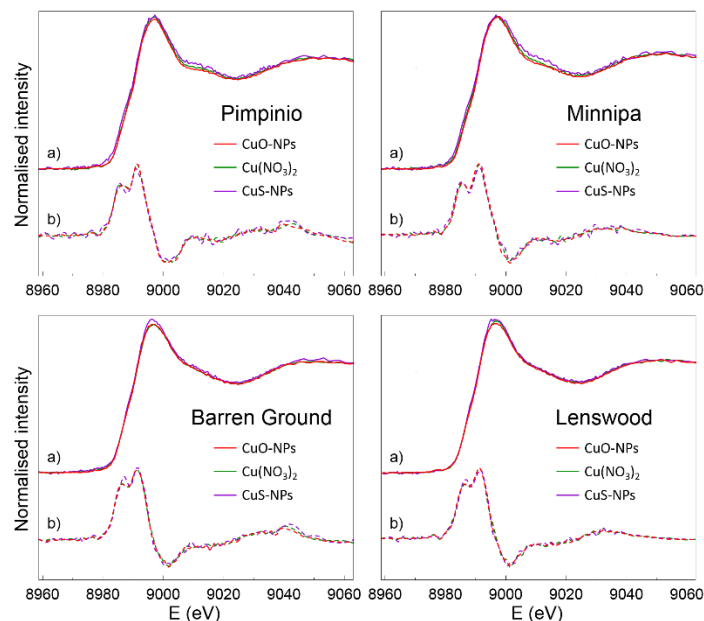
Figure 3. Cu K-edge XANES spectra of 4 different soils 4 months after spiking with CuO-NPs, CuS-NPs, or dissolved Cu(NO$_3$)$_2$. For each set of spectra (a) their derivative spectra (b) are also shown.

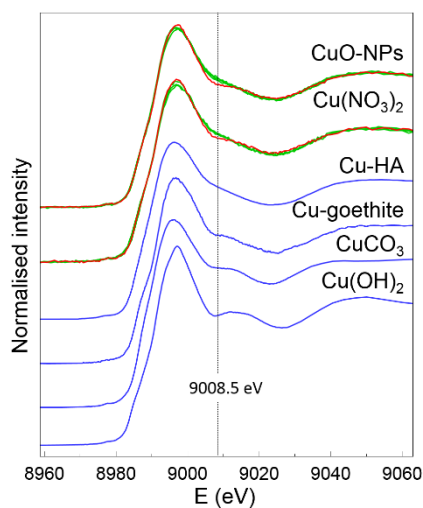**Figure 4.** (Red) Cu K-edge XANES spectra of CuO-NPs and Cu(NO$_3$)$_2$ added to Pimpinio and aged for 4 months, overlayed against (Green) spectra from other soils. (Blue) XANES spectra of Cu standards identified in the LCF. The dotted line at *ca.* 9008.5 eV indicates the inflection observed in the Pimpinio spectra, aligning with a feature commonly observed in Cu (II) bound to inorganic oxygen.

TABLES

Table 1. Selected properties of the soils used for $Cu(NO_3)_2$, CuO-NPs and CuS-NPs ageing. The extended range of soil parameters are available in Supplemental Table S1.

Soil name	Soil type	pH	WHC (%)	EC (dS/m)	N (wt %)	TOC (wt %)	Na (wt %)	Cu (mg/kg)
Barren Grounds	Organosol	5.0	26.5	0.03	0.22	5.48	0.0002	7.61
Lenswood	Kurosol	5.3	33.0	0.02	0.18	2.75	0.0010	3.91
Hamilton	Ferrosol	6.8	40.7	0.03	0.16	3.05	0.0132	1.09
Pimpinio	Vertisol (red)	8.0	43.7	0.22	0.14	1.27	0.0425	9.59
Minnipa	Calcarosol	8.0	40.7	0.12	0.11	1.48	1.8260	--

Table 2. Linear combination fitting (LCF) results of the Cu K-edge XANES spectra acquired from soils spiked with dissolved $Cu(NO_3)_2$ (top) and CuO-NPs (bottom). The LCF results are shown as percentage composition of the total Cu in terms of the listed standards. FeO(OH) indicates any ferric oxyhydroxide (in this case, Cu-Fh and Cu-Gt have been summed). Note that LCF contributions of <10% are not considered significant due to the inherent errors in the LCF procedure. They are only included here for completeness.

$Cu(NO_3)_2$	BARREN GROUND			LENSWOOD			HAMILTON			PIMPINIO			MINNIPA		
Components	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
CuO															
Cu-FeO(OH)	88.7	55.8	48.2	28.7	35.9	32.3	34	43.5	31.4					40.3	19.3
NOM†		38.2	46.2	39.3	62.6	61.8	45.4	37.1	54.2				47.8	21.5	50.9
$CuCO_3$										51.4	51.2	50.6		20.7	
Cu_2S				31.9	1.4	5.9	11.8	12.9	8.7	15	15.5	16.1	16.7	17.5	13.5
$Cu(OH)_2$							8.8	7.2	6.5	36.2	43.2	35.0	37.4		17.2
Cu-HA	11.3	6.2	6.1												
Day 0 fraction‡		54.7	54.3		4.3	18.4		81.7	73.5		52.5	64.9			45.9
SUM	100.0	100.2	100.5	100.1	99.9	100.0	100.0	100.7	100.7	102.6	109.9	101.7	101.9	100.0	100.9
R-factor	5.0E-04	3.8E-05	4.7E-05	7.0E-04	7.6E-05	1E-04	1.9E-04	1.6E-04	9.8E-05	8.8E-04	2.6E-04	1.9E-04	2.8E-04	3.7E-04	7.7E-05

CuO-NPs	BARREN GROUND			LENSWOOD			HAMILTON			PIMPINIO			MINNIPA		
Components	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
CuO (NPs)	73.2						83.9			81.2	41.1	26.5	97.1	60.7	46.2
Cu-FeO(OH)	22.9		40.8	24.3	33.5	28.4	13.7	39.1	40.7	17.6	39.4	60.7	4.3	20.9	28.9
NOM†		75.7	59.2	67.3	67.2	67.4		56.7	51.2		13.0			10.8	16.8
$CuCO_3$															
Cu_2S	5			8.4		4.2	5.4	4.2	8.1	3.2	6.4	14.0		7.7	10.1
$Cu_3(PO_4)_2$		4.0													
$Cu(OH)_2$		20.3													
Cu-HA															
SUM	101.1	100.0	100.0	100.0	100.7	100.0	103.0	100.0	100.0	102.0	99.9	101.2	101.4	100.1	102.0
R-factor	7.4E-05	7.8E-05	1.0E-04	4.7E-04	9.3E-05	1.2E-04	1.6E-04	1.0E-04	1.5E-04	2.2E-04	1.7E-04	2.8E-04	1.5E-04	6.7E-05	8.6E-05

† NOM is the sum of the percentage contributions from native SR-NOM and SR-NOM dialysed with $Cu(NO_3)_2$.

‡ Day 0 fraction is the percentage attributed by the LCF to the corresponding Day 0 spectrum before distributing to other components.

SUPPLEMENTARY INFORMATION

Ageing of dissolved copper and copper-based nanoparticles in five different

soils: short term kinetics vs long term fate

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Principal Component Analysis, Target Transformation and Linear Combination Fitting analysis procedures

Principal Component Analysis (PCA) and Target Transformation (TT) were performed using the SixPack program. PCA was run using all the sample spectra between 8970 – 9100 eV (-25 – 120 eV relative to Cu-K absorption edge of 8979 eV) and IND values indicated that up to 9 components were likely to be contributing to the sample spectra. TT classified the standard spectra according to the SPOIL values shown in Table S2 below, where 8 standards were classified as "good" (SPOIL <3.0) which were included in the Linear Combination Fitting (LCF). As a large number of components were indicated as likely by PCA, additional standards based on what may be likely for the soils were included in the LCF: Cu bound to HA, Suwanee River Natural Organic Matter (SR-NOM), Cu-goethite, $Cu_3(PO_4)_2$ and malachite were included based on what may be expected for the soils. LCF was performed using the Athena program with a maximum of four standards in each simulated combination. Combinations with five standards as a trial on several spectra returned mostly the same fits with four or less components, so four was decided as the maximum.

Synthesis of selected Cu standards

Cu sorbed to ferrihydrite

40 g of $Fe(NO_3)_3.9H_2O$ was dissolved in 500 mL of ultrapure water and 1 M KOH was slowly added under vigorous stirring. The pH was constantly monitored and the addition of KOH was stopped when the solution pH reached 7.23. The precipitate (ferrihydrite, Fh) was rinsed 5 times by centrifuging and re-suspending in ultrapure water before freeze-drying the sample. Cu-sorbed on to Fh was prepared by $Cu(NO_3)_2$ solution to Fh at a dose of 1000 mg Cu/kg, allowed to equilibrate for 24h on the orbital shaker in the dark, and dialysed for 48 h with ultrapure water to remove the excess, unadsorbed Cu. The pH of the Fh suspension at the point of Cu addition was 4.6. The final product was freeze-dried and collected and used as a Cu-ferrihydrite standard.

A similar procedure was followed with Cu sorbed to goethite using synthesized goethite.

Cu with organic ligands

Cu-aspartate was freeze-dried from a 20 mL solution of 222 mg/L Cu^{2+} (as $Cu(NO_3)_2$) with 50 mg aspartic acid. The pH of the final deep blue solution was 7.2 prior to the freeze-drying. The molar ratio of Cu to aspartate was ca. 1:5.

Cu-cysteine was prepared by mixing 444 mg/L Cu^{2+} (as $Cu(NO_3)_2$) with 50 mg cysteine. The colour of the solution was cloudy with a hint of pink. The pH was 2.05 prior to freeze drying. The molar ratio of Cu to cysteine was ca. 1:3.

Cu-EDTA, Cu-histidine and Cu-arginine were prepared in a similar manner, with a molar ratio of 1:3.

Supplemental Table S1. Properties of the soils used in this ageing study. This is an extended version of Table 1 of the main text.

| Soil name	Soil type	pH	WHC (%)	Clay (%)	Silt (%)	Sand (%)	EC (dS/m)	TOC (wt %)	TN (wt %)	Na (wt %)	Ca (wt %)	Fe (wt %)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Barren Grounds	Organosol	5.0	26.5	17.2	6.4	76.4	0.03	5.48	0.22	0.0002	0.03	0.25	7.2	7.61	9.3
Lenswood	Kurosol	5.3	33.0	17.6	12.4	70.1	0.02	2.75	0.18	0.0010	0.03	0.67	24.6	3.91	11.7
Hamilton	Ferrosol	6.8	40.7	16.0	16.4	67.7	0.03	3.05	0.16	0.0132	0.03	2.14	75.6	1.09	26.6
Pimpinio	Vertisol (red)	8.0	43.7	16.0	32.0	52.1	0.22	1.27	0.14	0.0425	0.43	2.28	254.1	9.59	22.6
Minnipa	Calcarosol	8.0	40.7	--	--	--	0.12	1.48	0.11	1.8260	1.08	0.56	0.003	--	0.01

Supplemental Table S2. SPOIL values resulting from the PCA and 9-component TT processes of the Cu standards. The first 8 standards with the low spoil values and additional selected standards were used for LCF analysis. For non-Day 0 samples, the Day 0 spectrum was included as a "standard" in the LCF analysis and redistributed after the analysis. [*1]Contributions from SR-NOM dialysed (13.5 kD) with water before freeze drying (SR-NOM/H_2O) and native SR-NOM were summed in Table 2 of the main text. [*2]CuS-NPs used only for CuS-NPs aged for 4 months (135 days).

Copper species	SPOIL	LCF
CuS (covellite)	1.5237	Yes
SR-NOM/Cu(NO$_3$)$_2$	1.5447	Yes[*1]
CuO-NPs	1.5567	Yes
Cu$_2$S	1.5914	Yes
CuO	1.7542	Yes
Cu-cysteine	1.8101	Yes
Cu(OH)$_2$	2.1369	Yes
Cu-ferrihydrite	2.7403	Yes
Metallic Cu	3.1392	No
Cu$_3$(PO$_4$)$_2$	3.4573	Yes
CuS-NPs	3.5888	Yes[*2]
Cu-histidine	3.5932	No
Cu-alginate	3.627	No
Cu-goethite	3.9691	Yes
Cu$_2$O	4.046	No
Cu-EDTA	4.748	No
Cu-aspartate	5.0345	No
Cu-oxalate	5.1802	No
Cu-HA	5.1846	Yes
SR-NOM	7.8584	Yes[*1]
Malachite	7.2875	Yes
CuCO$_3$	8.1773	Yes

Supplemental Table S3. Linear combination fitting (LCF) analysis of Cu K-edge XANES spectra of dissolved Cu (as $Cu(NO_3)_2$), CuO-NPs and CuS-NPs aged for 4 months (135 days) in soil. Note that LCF contributions of <10% are not considered significant due to the inherent errors in the LCF procedure. They have, however, been listed for completeness.

Components	BARREN GROUND			LENSWOOD			PIMPINIO			MINNIPA		
	$Cu(NO_3)_2$	CuO-NPs	CuS-NPs	$Cu(NO_3)_2$	CuO-NPs	CuS-NPs	$Cu(NO_3)_2$	CuO-NPs	CuS-NPs	$Cu(NO_3)_2$	CuO-NPs	CuS-NPs
CuO (NPs)	7.4									2.8	13.4	5.3
CuS (NPs)												
Cu-FeO(OH)	77.3	93.7	95.8	93.8	90.4	96.6	95.8	84.7	78.4	72.4	78.0	53.2
NOM*[1]	7.1	8.8	8.5	9.6	12.2	7.7			20.7	27.6	10.4	46.7
$CuCO_3$												
CuS												
Cu_2S												
$Cu(OH)_2$							7.2	17.5	5.6			
Cu-HA	9.9											
SUM	101.7	102.5	104.3	103.4	102.6	104.3	103.0	102.2	104.7	102.8	101.8	105.2
R-factor	1.0E-04	6.4E-04	7.8E-04	6.2E-04	4.9E-04	6.6E-04	3.2E-04	2.5E-04	6.2E-04	3.6E-04	2.6E-04	7.6E-04
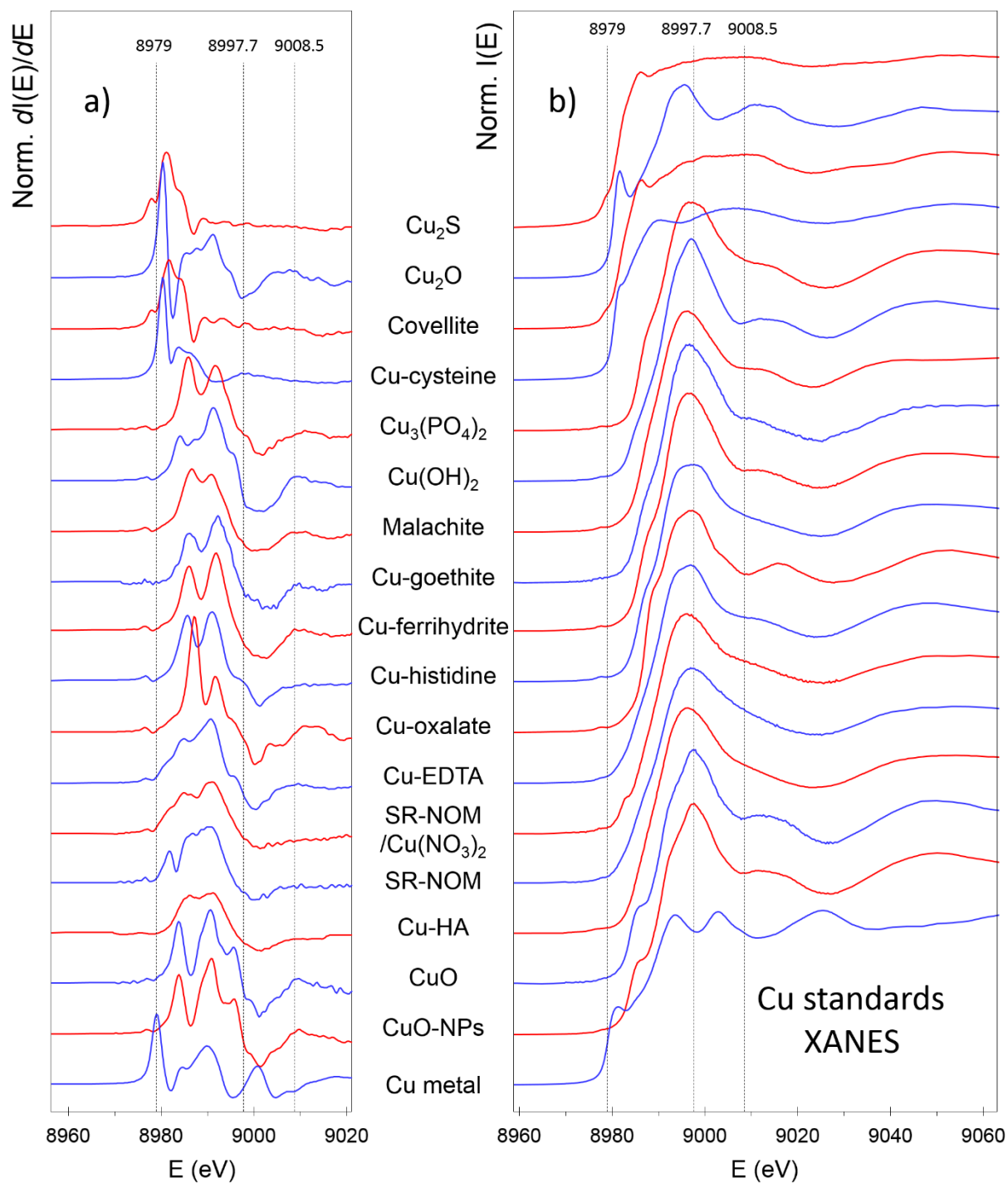
Supplemental Fig. S1. K-edge XANES spectra of selected copper standards: a) derivative spectra, dI(E)/E, and b) normalized intensity, I(E). The energy was calibrated using the first inflection point of the Cu metal 1st derivative spectrum (8979 eV, left dotted line). The white line of the CuO-NPs is at 8997.7eV indicated by the dotted line (right). The right dotted line (9008.5 eV) indicates the inflection feature common across standards with Cu (II) bound to an inorganic oxygen atom. Note that Cu-oxalate also show similar feature but its energy is slightly higher.
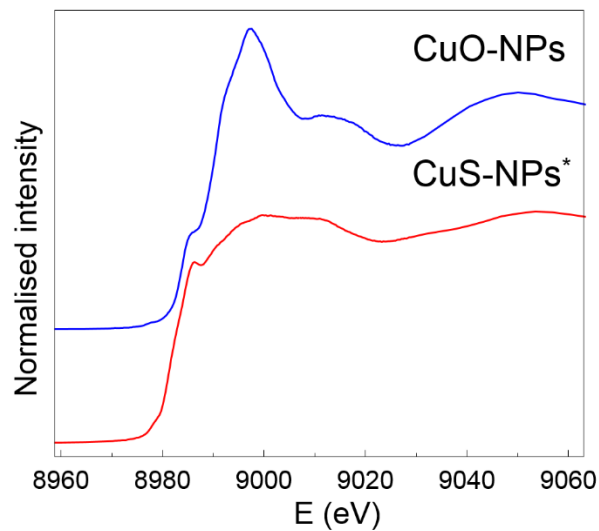
Supplemental Fig. S2. Cu K-edge XANES spectra of CuO and CuS nanoparticles. *CuS-NPs are immobilised on Kapton film via plasma polymer and shows slight sign of oxidation.
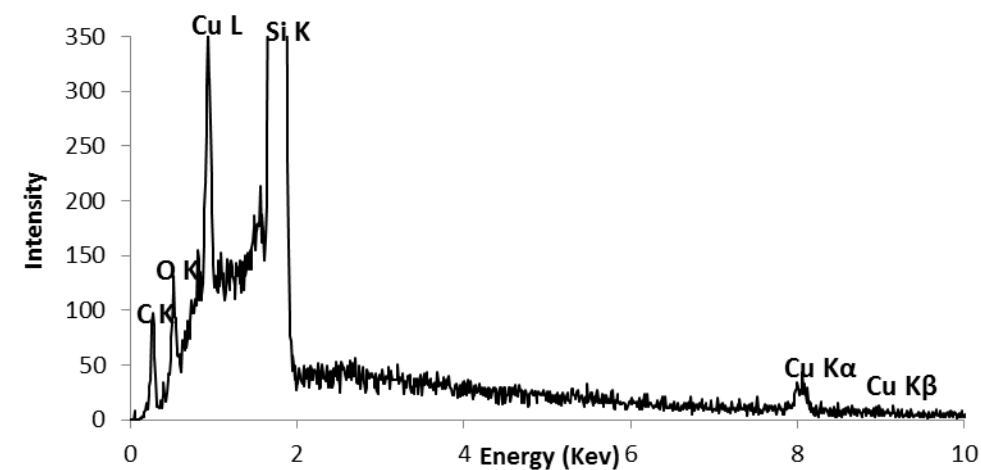
Supplemental Fig. S3. Energy dispersive X-ray spectrum of the CuO-NPs confirming the elemental composition (Background Si signal is from the wafer on to which the CuO-NPs were deposited for EDX analysis).