**Editorial comments:**  
  
1.The Long Abstract reads like an original research article; it should focus more on the methods. – ***We have tried to alter the long abstract to include more about the abstract and incorporate major concerns from viewer number 3.***  
  
2. Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.   
  
3. Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.   
  
4. If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.   
  
**Reviewers' comments:**  
  
**Reviewer #1:**   
*Manuscript Summary:*   
The manuscript is an outstanding contribution from the authors in the area of CuO-NPs treatment to effectively remove contaminants from PBW and reduce mammalian cytotoxicity.  
  
*Major Concerns:*  
Cell starvation questions has been successfully assessed by measuring growth factor concentrations in the media periodically during treatment. This technique has future scope of research as well.-- ***Added a comment on line 684 including cell starvation as possible future work.***  
*Minor Concerns:*  
Disposal of wastes can be addressed better by the technique. – ***Disposal of used CuO-NPs is addressed through the regeneration process of CuO-NPs by other studies (Reddy et al., 2013), which is referenced in the paper. Regeneration of CuO-NPs is addressed in lines 127-130 and 683.***  
  
**Reviewer #2:**   
*Manuscript Summary:*   
This is a well-written paper that reports important results on the use of copper oxide nanoparticles to remove contaminates from uranium mine production bleed water and the effect on mammalian cells. While I believe it could be published in its current form, I believe it would be improved with minor revision and have recommended a few items for the authors to consider in their final revision.  
  
*Major Concerns:*  
No major concerns.  
  
*Minor Concerns:*  
No minor concerns.  
  
*Additional Comments to Authors:*  
On line 115, the authors use the term "CuO-NPs" without definition (although it is in the abstract, it should be given here as well to allow the paper to stand alone). The definition is provided on line 146, but that should be moved to line 115.—***CuO-NPs was defined on line 115***  
  
In line 169, the authors use the term "10x EMEM". What is this? Later, this is identified as a cell growth or culture media, but this should be identified at the first use. Since this is not a cellular biology journal, I recommend that a few words of explanation be added at the first use of specialized terms such as this to make the paper/videos more accessible to the broader audience targeted by this journal. – ***Additional descriptions of EMEM and how it is used have been added.*** On line 289 (and used again in 294, 296, and 300), the acronym "PBS" is introduced without definition. (Could this be misspelled and actually represent fetal bovine serum, FBS? My technical definition of PBS is polybutadiene-styrene plastic, but as it is later indicated to be a liquid, I suspect that this is not the authors' intended meaning.) If it is not misidentified, please define PBS and add it to the list of chemicals in the Table of Materials/Equipment. – ***PBS is phosphate buffered saline and has been defined on line 306 and has been added to the Table of Materials/Equipment.*** Similarly for DMSO on line 316: while I am confident that this is dimethyl sulfoxide, the authors should not leave their readers/viewers guessing.—***DMSO is now defined as dimethyl sulfoxide on line 334. Please note lines have shifted due to edits in previous sections.***  
"Pellets" are referenced in lines 189, 192, and 194-195. Are these the precipitated CuO particles***? – The term pellets has been replaced with CuO-NPs.***    
  
In line 530, "charged" is misspelled as "charge". – ***This has been corrected***  
  
The upper graph in Figure 1A contains a black line, which appears to correspond to the 16.5% yellow bar in the lower graph. Should this line be yellow, like the one in the upper graph of Figure 1B? – ***The line in figure 1A has been changed to yellow to match the line in Figure 1B.***

In Tables 1 and 2, up to 5 significant figures are given. I suspect that the accuracy of the analyses does not justify this level of reported precision. I recommend that the table be edited to include the appropriate number of significant figures (perhaps 3?) for each reported value. -- ***Significant figures were adjusted.***  
  
**Reviewer #3:**   
*Major Concerns:*  
No major concerns. In general a well developed experimental protocol that melds aspects of geochemistry and ecotoxicity together to produce an engaging study of the application of CuO nanoparticles. I would have liked to see more prominent in the text (and also mentioned in the Abstract) that this in vitro study is limited in its wider context due to dilution effects and changes in pH from traditionally alkaline leach of the PBW. This is just to better place this study in context. –***We have tried to adjust the abstract to reflect these concerns as well as the editorial concerns that it doesn’t focus on the methods.***  
  
Some more information on the nature of the CuO-NPs would have also been very useful. This is possibly contained in some other related publications but information such as the particle size distribution, presence or not of aggregates and some SEM images of the particles would have provided the reader with some more (and essential) basic information on the nature of the absorbent medium. ***– This study did not measure the attributes of the CuO-NPs directly so we only refer to those numbers from Martinson and Reddy, 2009.***  
*Minor Concerns:*  
Sometimes abbreviations are used and other times not. Use abbreviation after the first mention and thereafter. –***We corrected this issue.***  
I still do not know what EMEM is after reading the manuscript twice. – – ***Additional descriptions of EMEM and how it is used have been added*** Check significant figures in tables. – ***Significant figures in tables have been reduced.***  
Figures are inanimate so cannot "show" something or be used at the beginning of a sentence. This is commonplace in manuscripts but still is incorrect irrespective of how common an error that it is. – ***These have been corrected in the text.***Could have been a more general summary of U and contaminant removal techniques in the introduction. Just a few more lines and key references to round things out a little more. – ***More was not added about Uranium and the removal techniques in order to expand upon the methods and results. If addition of this information is recommended by the editors it can be added.***

Please see the attachment – ***Comments in text have been addressed with track changes in text.***  
  
*Additional Comments to Authors:*  
None other than specific comments marked in the text. – ***Comments in text have been addressed with track changes in text.***