**Editorial comments:**  
1. Please state the goal of your protocol in the Short abstract. Please ensure not remain within the 10-50 words limit.

*The goal of this protocol was to integrate the native environmental sample into a cell culture format assessing the changes in toxicity due to CuO-NP treatment.*  
  
2. Please re-write steps of your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.). For instance, in step 1.1, “Collect two types of water samples..” and mention   
PBW is wastewater…” as a NOTE below the step.

*We have rewritten the protocol section in the imperative tense. Step 1.1.1 added PBW is wastewater…” as a NOTE below the step*

3. Please try to avoid usage of phrases such as “should be”, “could be”, “would be” and write in the active/imperative style. For instance in step 1.2.

*All uses of the phrases “should, could or would be” have been removed from the protocol section. They still exist in some instances in the text. If you would like me to rephrase those sections please let me know.*  
4. Please make sure the entire protocol section is written in imperative tense. “Place the solution..”, “Allow the solution to cool…”. “Allow the pellets to dry..”, “Scrape the pellets out…” etc.

*Hopefully I have corrected this.*  
  
5. Please mention the centrifugation speed and time in step 3.2. and where needed.

*Step 2.4 added speed and time, Step 3.2 added speed and time, more explanation added to step 7.3 including centrifugation speed and time*  
6. Please replace step 5.2 as a note. Additionally please mention how to obtain Visual MINTEQ.

*5.2 has been replaced with note 5.1.2, The link for downloading visual MINTEQ has been added.*  
  
7. Please revise the text to avoid the use of any pronouns (i.e. "we", "you", "your", “our” etc.). If you feel it is very important to give a personal example, you may use the royal "we" sparingly and only as a "NOTE:" after the relevant protocol step. Please use the Ctrl+F function to find and replace the pronouns.

*We have left only two instances of our in the text, there are none within the protocol. If you believe we need to change these pronouns I will rewrite those statements as well.*  
  
8. References are not in correct JoVE format. Please make sure that your references comply with JoVE instructions for authors. In-text formatting: corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text of the manuscript. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI, (YEAR).]

*We believe the in-text formatting of the reference numbers to be correct. If this is still incorrect please let us know.*

We apologize, we downloaded the Zotero JoVE format and didn’t double check that it formatted correctly. Apparently the format listed on the site is incorrect and we don’t know how to download the csl file from the link you have listed on the JoVE website. We have manually corrected the following:

*#16, 21, 30, 32, 37, and 39 had two names listed plus et al for more than 6 references. We have removed the second name.*

*#20, 26, 34, 48 had no DOI #*

*We have removed the second initial from all of the reference*

*We have double checked that all references have page numbers.*

We do not know how you would like websites references formatted. Please let us know how you would like references 1-9 formatted.

Thank you for your time and help,

Jodi Schilz

KJ Reddy