Author's response to reviews

Title: Cell type specificity of lung cancer associated with low dosage soil heavy metals contamination in Taiwan: An ecological study

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Author's response to reviews: see over
Dear Ms Audrey Ann Reyes,

Thank you very much for your assistance regarding our manuscript. We have carefully addressed yours and the reviewers’ comments, and have incorporated all the suggestions in the revised manuscript. In addition, we have followed the editorial requirements for the journal. If you have any questions regarding the revisions, please contact me. Once again, thank you very much for your assistance.

The point-by-point responses to the editor's and reviewers’ comments are as follows:

**Editor's comments**

Q1: Please document within the methods section of your manuscript the specific name of the organization that granted ethical approval to your study going ahead.

A1: The data obtained for this study are open to public.

Q2: Figure cropping.

A2: The arrangement of those figures has been modified to fit the best use of the spacing.

    Please see figure files 1 and 2.

**Reviewer’s comments**

For Reviewer: Rafael Lefkowitz

Minor essential revisions

Q1: Page 1, line 5 and page 5, line 80: Replace “especial” with “especially”.

A1: Replacement made. Please see line 5 of page 1 and line 79 of page 5.
Q2: Page 1, line 5: “incidence rate” should be corrected to “incidence rates” (plural).

A2: Correction made. Please see line 12 of page 1.

Q3: Page 1, line 19: correct “of trend test” to “of the trend test”.

A3: Correction made. Please see line 19 of page 1.

Q4: Page 2, line 21: correct “have significant” to “have a significant.”

A4: Correction made. Please see line 21 of page 2.

Q5: Page 3 line 35: Sentence starting with “The heavy…” needs grammatical revision.

A5: “The heavy…” has been rephrased to “The paths of heavy metal circulation in the environment that human beings are directly exposed to are those via air, drinking water, and food.” Please see line 35-37 of page 3.

Q6: page 3 lines 40 -41: “is higher, are prone to be exposed to the air,” correct to:

“is higher are prone to be exposed to air”

A6: Correction made. Please see line 40-41 of page 3.

Q7: Page 4, line 51: “And those result in” correct to “This is hypothesized to result in”

A7: Correction made. Please see line 51 of page 4.

Q8: Page 4, line 5: “But only few studies” correct to “But there are only a few
studies”.

A8: Correction has been made as follows: “however, only a few studies on Pb and lung cancer were addressed.” Please see line 55-56 of page 4.

Q9: Page 4, line 57. Change “mercury” to “mercury (Hg)” and in line 59 replace mercury with Hg and then use the abbreviation thereafter.

A9: Modification made. Please see line 58 of page 4.

Q10: Page 4, line 67: “disease map is” correct to “disease maps are”.

A10: Modification made. Please see line 66 of page 4.

Q11: Page 4, line 65: Sentence beginning with “The above studies…” is superfluous.

A11: The sentence has been deleted.

Q12: Page 4, line 68: “casual” authors intend “causal” (wrong word).

A12: The term “a causal relationship” has been modified to “an association” according to the suggestion from the other reviewer. Please see line 67 of page 4.

Q13: Page 5, line 72: “of the areas” authors intend “of certain areas”?

A13: The authors intended “of the corresponding studied areas”. Please see line 71-72 of page 5.

Q14: Page 5 line 72-77: Redo this sentence.

A14: The sentence has been rephrased as “Another study in the US found that the spatial
variation pattern of smoking prevalence and lung cancer was similar [26] when comparing the temporal and geographic variation of lung cancer incidence for both white males and females during 1950 to 1994 and the smoking prevalence for both males and females in 1985.” Please see line 72-75 of page 5.

Q15: Page 5 line 77: “strengthen” correct to “strengthening”
A15: Correction made. Please see line 76 of page 5.

Q16: Page 6 line 98: “by Taiwan” correct to “by the Taiwan”.
A16: Correction made. Please see line 104 of page 6.

Q17: Page 6, line 99: “population 12 groups” correct to “12 population groups”.
A17: Correction made. Please see line 105 of page 6.

Q18: Page 7 line 115 “by descending and” correct to “by descending order and”.
A18: Correction made. Please see line 121 of page 7.

Q19: Page 7, line 116: “significant difference of the” authors intend “significant difference from the”?
A19: The authors intended to bring up the significant geographical difference of the standardized incidence rates which can be layered to 7 levels.

Q20: Page 8, line 140: “exist” correct to “is”
A20: Correction made. Please see line 154 of page 9.
Q21: Page 8, line 140: “areas, there” correct to “areas, and there”
A21: The sentence has been rephrased as “areas; the Cd concentration is sporadically higher in northern and central Taiwan.” Please see line 154-155 of page 9.

Q22: Page 8, line 141: Redo sentence starting with “There is…”
A22: The sentence has been rephrased as “Townships having higher Cr concentration are in western and northeastern coastal areas.” Please see line 155-156 of page 9.

Q23: Page 8, line 144: Is nickel concentration intended as plural (“were higher”)?
A23: The statement has been rephrased as “Ni concentration is higher in the western and southeastern coastal townships.” Please see line 158-159 of page 9.

Q24: Page 9, line 147: “1982-1986, the” correct to: “1982-1986. The…” (Start new sentence.)
A24: Correction made. Please see line 161 of page 9.

Q25: Page 9, line 163, and page 10 line 164, and page 10 line 165, and page 10 line 167: “has cluster” authors intend “is clustered”?

Q26: Page 9, line 169 and page 11 line 191: “adjusted the gender” correct to “adjusted for the gender”.
A26: Correction made. Please see line 183-184 of page 10.
Q27: Page 10, paragraph starting line169: Recommend consistency in the style of presenting RR and 95%CI.

A27: Modification made. Please see paragraph starting at line 190 of page 11.

Q28: Page 11, line 188: “and Zn have statistical significant on dose-response relationship”.
Authors intend “and Zn indicates a statistically significant dose-response relationship.”?


Q29: Page 11, line 194: “statistically significant concentration” authors intend “statistically significant finding”?  

A29: Yes. Modification made. Please see line 208 of page 12.

Q30: Page 11, line 197: “Those reaches a statistical significance” authors intend “Those that reach statistical significance”? 

A30: Yes. Modification made. Please see line 211 of page 12.

Q31: Page 11 line 199: “and are Cu” correct to “and Cu”.

A31: Correction made. Please see line 213 of page 12.

Q32: Page 12 line 209: “to conduct the association” It appears there are missing words.

A32: Correction made. Please see line 223-224 of page 13.

Q33: Page 12, line 212: “either male or female” correct to “either men or women”.

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A33: Correction made. Please see line 226 of page 13.

Q34: Page 12, line 212: “rate have spatial cluster” correct to “rates are spatially clustered”.
A34: Correction made. Please see line 226 of page 13.

Q35: Page 12, line 214: “potential environmental risk” correct to “potential for environmental risk”.
A35: Correction made. Please see line 227-228 of page 13.

Q36: Page 12, line 218: Sentence beginning “The interesting finding..” Authors intend “The interesting finding of this study is that for some heavy metals, soil concentrations at levels lower than regulatory standards are associated with lung cancer incidence.”?
A36: Yes. The sentence has been replaced by “The interesting finding of this study is that for some heavy metals, soil concentrations at levels lower than regulatory standards are associated with lung cancer incidence.” Please see line 232-234 of page 13.

Q37: Page 13, line 221: “still show significant” correct to “still show a significant”.
A37: Correction made. Please see line 235 of page 13.

Q38: Page 13, line 228: “higher Cr concentration” please clarify the Cr source –soil chromium, blood chromium, other?
A38: The statement has been clarified by rephrasing the sentence as “the exposure to higher Cr concentration in workplace”. Please see line 242-243 of page 14.

Q39: Page 13, line 231-232: “relatively lower” Authors intend “relatively lower than
regulatory standards”? Please clarify.

A39: The author intended “relatively lower than TEPA guideline. Please see line 246 of page 14.

Q40: Page 13, line 237: “both gender. I.e. Subjects” correct to “genders, i.e. subjects”

A40: Correction made. Please see line 251 of page 14.

Q41: Page 13, line 238: “pathology” correct to “pathologies” (plural).

A41: Correction made. Please see line 252 of page 14.

Q42: Page 14, line 240 and line 248: “gender” correct to “genders”.

A42: Correction made. Please see line 255 of page 14.

Q43: Page 14, line 241: “Ceruloplasmin” should not be capitalized.

A43: Correction made. Please see line 257 of page 14.

Q44: Page 14, line 247: “has positive” correct to “has a positive”.

A44: Correction made. Please see line 262 of page 15.

Q45: Page 14, line 256: “in nickel factory than normal population” authors intend “in nickel factory workers than in the normal population”?

A45: Yes. Correction made. Please see line 271 of page 15.

Q46: Page 14, line 257: “exist” authors intend “exhibit”?

A46: Yes. Correction made. Please see line 272 of page 15.
Q47: Page 15, line 259: “has” correct to “have” (plural).

A47: Correction made. Please see line 274 of page 15.

Q48: Page 15, line 159-260: “Ni concentrations” please clarify the Ni source
(blood?)

A48: The source of Ni concentrations is from lung biopsy specimen indicated in reference [44], Kuo et al. The sentence has been rephrased as “Ni concentration in lung biopsy specimen”. Please line 274 of page 15.


A49: Correction made. Please see line 275 of page 15.

Q50: Page 15, line 264: “gender” correct to “genders” (plural).

A50: Correction made. Please see line 279 of page 16.

Q51: Page 15, line 264: “susceptibility of Ni to lung SCC than to lung AC” authors intend “propensity to be associated with lung SCC than with lung AC”?

A51: Yes. Correction made. Please see line 280 of page 16.

Q52: Page 15, line 275: “the Zn concentration” please clarify the Zn source
(blood)?

A52: Zn concentrations are from serum in reference [21], Diez et al. The sentence has been rephrased as “serum Zn concentration”. Please line 291 of page 16.
Q53: Page 16, line 283: “smoke prevalence” correct to “smoking prevalence”.

A53: Correction made. Please see line 299 of page 17.

Q54: Page 17, line 303: “townships; However, the” correct to “townships; however, the” (no capital H if it is not starting a new sentence).

A54: Correction made. Please see line 327 of page 18.


A55: Correction made. Please see line 337 of page 19.

Q56: Figure 2a-d. I would suggest against using 2 similar shades of yellow for regions with very different incidence rates.

A56: The figures were re-built under the consideration provided by the reviewer. Please see figure 2.

Q57: Page 7, line 108: Number of townships here is 283, but on page 9 line 146 it is 285. Please clarify for consistency.

A57: The data of the eight metals were collected from 285 townships in the first place. However, since there are data missing on metal As in two of those townships, the analyses throughout the entire study were based on 283 townships. The sentence beginning from line 144 of page 8 has been rephrased as “Table 1 shows the median concentrations of those eight soil heavy metals according to data of 283 townships (N=283).” The text description on table 1 has also been modified to make our intention clear. Please see line 160-161 of page 9 and table 1 on page 30.
Major compulsory revisions

Q1: Page 5 line 79: The authors note some studies have discussed the relationship between lung cancer incidence and soil heavy metal contamination. Please cite appropriate references.

A1: The authors intended to express that “few studies” have discussed the issue instead of “fewer studies”. Please line 78 of page 5.

Q2: Page 12, lines 203-204: I would like the authors to clarify use of reference 29 in support of this statement. After reading the cited reference (Goldsmith, DF: Calculating cancer latency using data from a nested case-control study of prostatic cancer. J Chronic Dis 1987, 40(Suppl 2):199-123), it is unclear how the authors intended to relate this article to their statement on lung cancer latency. Please clarify the reference for this statement, or state another reason for using 15 year latency.

A2: After carefully considering the estimation, we determined the lung cancer latency to be around 15 years by referring to another study in reference 23 --- Colye YM, Minahjuddin AT, Hynan LS, Minna JD: An ecological study of the association of metal air pollutants with lung cancer incidence in Texas. J Thorac Oncol 2006 Sep 1(7):654-661. Please see line 218 of page 12 and line 426 of page 23.

Q3: Page 16, line 295-297: I am confused- are the authors stating here that in this article soil heavy metal concentrations are used as exposure indicators? This is inconsistent with the Background. A statement consistent with the Background would be “soil heavy metals are often used as exposure indicators” (the word “often” is added).

A3: Modification made. Please see line 312 of page 17.

Q4: Page 17, line 297-298: Provide additional detail regarding how concentrations of air pollutants were used to adjust the regression model and include this in the methods section.

A4: Data of concentrations of air pollutants during 1994-1998, acquired from Taiwan Air Quality Monitoring Network operated by TEPA, were introduced to the analyses. There were only 48 townships in this data having records of soil heavy metal concentrations. The mean concentrations of CO, NO, NO₂, O₃, PM₁₀, and SO₂ among these 48 townships were used to adjust the regression model. The medians (quartiles Q1-Q3) of
these air pollutants are CO 0.61ppm (0.53-0.82), NO 6.82 ppb (4.28-10.35), NO$_2$ 20.43 ppb (16.40-25.26), O$_3$ 22.37 ppb (20.76-25.66), PM$_{10}$ 62.89 µg/m$^3$ (50.06-77.00), and SO$_2$ 5.34 ppb (3.31-6.60). Please see line 140-148 of page 8 and 9.

After we applied the adjustment of air pollutants on the analyses, the significance showed in RR trend tests for the following analyses have changed: lung AC and Cu, Ni in males, lung AC and Cu, Zn in females, lung SCC and Cr, Cu, Hg, Ni, Zn in males, and lung SCC and Cr, Ni, Zn in females. The significance were only exhibited in lung AC and Cu for females and lung SCC and Cr, Ni, Zn in males, whereas non-significance for the rests. Although this change may be due to the reduced statistical power, the stronger relationships between lung AC and Cu in females and between lung SCC and Cr, Ni, Zn in males were established. Please see line 314-322 of page 17 and 18.

**Discretionary Revisions**

**Q1:** I would consider using an additional figure, overlaying the cancer incidence data on the soil heavy metal data. This may depict the significant associations in a simple manner.

**A1:** We appreciate the suggestion. However, with the current software in our group, it is not possible to provide the figure. We will be preparing it for future researches.

**Q2:** Consider additional comment on the significance of the correlating soil metal contamination and how it may impact interpretation of positive trends between multiple soil metals and cancer incidence.

**A2:** We found that there are five metals including Chromium, Copper, Mercury, Nickel, and Zinc are associated with lung cancer in multiple regression analysis. Therefore, we categorized townships by number of metal types mentioned above that have concentrations exceeding Q3 into 4 groups. They are None of 5 metals exceeds Q3, Either any 1 or 2 of 5 metals exceed Q3, Any 3 of 5 metals exceed Q3, and Either any 4 or 5 of 5 metals exceed Q3. The result is attached at the end of this document. Regardless of pathological types of lung cancer, dose-response effect and increasing RR values were shown when the number of metal types exceeding Q3 becomes greater. We didn’t include the above information in the manuscript since few literatures pointed the effect of the combination of several heavy metals.

**Q3:** A discussion of the land-use history of highly contaminated regions would be greatly
informative. This may also be relevant to a discussion of correlating soil metal concentrations.

A3: We thank your precious advice. The land-use history and the type of a region are indeed worth discussing. However, we had no access to the relative information in this study. We aim to further explore the issue in the future.

For Reviewer: Hong Zhang

Minor Revision

Q1: I feel that authors raised an interesting question and well defined it. I just have a minor question for the topic chosen: Adenocarcinoma and squamous are both belong to non-small cell ca; Lung ca is clinically commonly categorized into small cell and non-small cell ca. It would be more expected to study small cell vs. non-small cell ca than comparing two cell types in the same category.

A1: According to Cancer Registry Annual Report in 2003 from Bureau of Health Promotion, Taiwan [27], 26.49% and 34.73% of lung cancer cases among males were reported as lung AC and SCC respectively (12.06% for small-cell carcinoma); among females, 9.50% and 61.84% of lung cancer cases were reported as lung AC and SCC respectively (3.51% for small-cell carcinoma). As compared to small-cell carcinoma, lung AC and SCC together apparently make up a larger portion among all types of lung cancer in Taiwan. Therefore, this study focused on lung AC and SCC over other types. Please see line 86-93 of page 5-6.

Q2: The method session: what are the selection criteria for townships if not all township selected? The numbers of township didn’t add up. Were the 354 townships obtained from National Cancer Registry Program all townships in the areas if study? Soil Heavy metal collected from 283 townships were stated in line 88 but the table 1 showed N= 285. In the limitation session, author stated “the date from only 40 townships were (was) left”.

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A2: The data of the eight metals were collected from 285 townships in the first place. However, since there are data missing on metal As in two of those townships, the analyses throughout the entire study were based on 283 townships.

Q3: In line 67 and 68 “In epidemiology, disease map is commonly used to explore the disease variation and then to generate the hypothesis of a causal relationship between disease and environmental factors”. “Disease map” maybe a direct translation from Chinese but I’m not sure is a common term in epidemiology, and consider the term “association” rather than a “causal relationship” since a spot map can not generate causal relationship.

A3: Disease map is a term commonly used in epidemiology. We have adjusted the term “a causal relationship” to “an association”. Please see line 67 of page 4.

Q4: Was the date used “incidence” or “prevalence”? It was mentioned as incidence as well as “occurrence” (Result session in Abstract).

A4: We intended “incidence”.

Q5: Similar as my own draft publications, there are many Chinese-English expression such as in Abstract line 5 and 6, “… especial(ly) for low dose heavy medical contamination exposure from soil”- consider the sequence of words like “from exposure to soil heavy metal contamination”… There are also many “is” should be past tense “was”. I would suggest an English editing before publication.

A5: The expression has been re-stated as “especially for exposure to low dose heavy metal contamination in soil”. Please see line 5-6 of page 1. On the other hand, the manuscript has been copyedited for English usage. Please see the certificate issued by Wallace.
Q6: The statistic method used it proper for this kind of study but I wasn’t able to get more time to study in detail.

A6: Thanks for the comment.

Q7: Overall, the article appeared to be high in internal validity, as well as external validity.

A7: Thanks for the comment.
Number among Chromium, Copper, Mercury, Nickel, Zinc concentrations > Q3 in the same township in relation to lung cancer risk

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<th>Level of the number</th>
<th>Squamous-cell carcinoma</th>
<th>Adenocarcinoma</th>
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<td>95% CI</td>
<td>P-value</td>
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<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4.5(# = 28)</td>
<td>1.37</td>
<td>1.25-1.50</td>
<td>&lt;0.01</td>
</tr>
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<td>1.29</td>
<td>1.16-1.43</td>
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<td>1.04-1.19</td>
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<td>-</td>
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<td>1.03-1.59</td>
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<tr>
<td>0(# = 126)</td>
<td>1</td>
<td>-</td>
<td>0.01</td>
</tr>
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</table>

# : number of townships in the specified category