

RESPONSE TO REVIEWER COMMENTS ON MANUSCRIPT: Investigating the synergistic potential Si and biochar to immobilize soil Ni in a contaminated calcareous soil after *Zea mays* L. cultivation Egusphere-2023-2687

The authors would like to thank anonymous reviewers for their time, invaluable comments and suggestions for substantially improving this manuscript. Please find detailed responses to each comment below.

ALL CHANGES ARE INDICATED IN GREEN HIGHLIGHT IN THE REVISED MANUSCRIPT

ANONYMOUS REFEREE #1

- 1. L. 61: Please, change “by product” by “byproduct” or “by-product”**

Authors’ response: We have made the correction as suggested by the reviewer.

- 2. L. 100: “... various physicochemical soil properties”. Please, describe which physicochemical soil properties were determined, and the methodology (with references).**

Authors’ response: We have added a concise description of each of the methods used to determine the soil properties with references, as also suggested by Reviewer 2.

- 3. L. 102: Please, add a brief description of the Boostani et al. (2023c) method. Was the soil incubated with Ni(Cl₂)? How long, under what conditions (temperature, darkness, moisture)?**

Authors’ response: We have added a concise description of the methodology as suggested by the reviewer, as also suggested by Reviewer 2.

- 4. L. 108: Please, briefly describe which chemical characteristics were assessed in biochar, and the methodology (with references).**

Authors’ response: We have added a concise description of each of the methods used to determine the biochar properties with references, as also suggested by Reviewer 2.

- 5. L. 137: Please, recalculate and replace “r.p.m.” by “x g”**

Authors’ response: We have recalculated and corrected it as suggested by the respectable reviewer.

- 6. Table 2: The Ni total content in soil prior to experiments is missing, i.e., the original Ni total content of the soil.**

Authors’ response: We have added the soil total Ni in Table 2 as suggested by the respectable reviewer.

- 7. L. 188: FTIR and SEM analysis should be in Material and Methods**

Authors’ response: We have added a concise description of the biochar properties that were determined including FTIR and SEM to the Materials and Methods section as suggested by the reviewer.

- 8. L. 192: It’s Table 3, isn’t it?**

Authors’ response: We have corrected the Table number as pointed out by the reviewer.

- 9. L. 205: It’s Figure 2, isn’t it?**

Authors’ response: We have corrected the Figure number as also pointed out by Reviewer 2.

10.L. 231: Why was the soil pH not measured after the experiments? If possible, determine and add to the manuscript these data to support your hypothesis.

Authors' response: Soil pH was measured after the experiments, as the correlation between soil pH and was stated in Line 226 of the original manuscript. We have added the soil pH data to the manuscript **supplementary information** as requested by the reviewer.

11. Same comment for L. 283 – 284 (“It has been shown that the application of Si to cultivated soils resulted in a reduction of soil organic matter content”).

Authors' response: Unfortunately, it is currently not possible to measure the amount of soil organic matter.

12.L. 232: Sachdeva et al., 2023: Full citation missing in the reference list.

Authors' response: This reference was added to the reference list.

13. Figure 4: Please, revise the Y-axis “Cd-Car”

Authors' response: The Y-axis of Fig. 4 has been corrected to Car-Ni as also pointed out by Reviewer 2.

14. Why was the Ni-Car fraction represented in a separate Figure and not with the other values in Table 4?

Authors' response: This is because, unlike the other soil Ni fractions, there was not a significant statistical interaction effect between biochar treatments and Si levels on the Ni-Car fraction. Therefore, the main effects of biochar and Si levels were shown separately in Figure 4 a and b, respectively.

15.L. 262: If possible, add a reference which justified the hypothesis “the decrease in the concentration of Ni in the carbonate ... Ni²⁺ ions in the soil solution”

Authors' response: We have added a reference as suggested by the respectable reviewer.

16.L. 273 – 278: “Lu et al. (2017) explored ... rate of the biochar”. What do you mean by this study? It is not very clear in the text, as they compare two types of biochar, but both of plant origin, whereas in the manuscript the comparison is between manure biochar and rice husk biochar. Furthermore, no specific (and related) conclusions of the study are given, only general information.

Authors' response: Thank you for pointing out this error. We have added the relationship of the previous studies results with the results of our study, and we have added a conclusion for the study.

17.L. 278 – 282: “In another study, the application ... control soil (Boostani et al., 2018)”. The results obtained in this study are very interesting to discuss your results. However, the results obtained in this manuscript were not specifically compared and discussed with this study.

Authors' response: We have now specifically compared and discussed the results of the Boostani et al. (2018) with our results as suggested by the respectable reviewer.

18.L. 289 – 290: Why was the MnOx-Ni fraction more affected by lower T biochars than higher T biochars?

Authors' response: A discussion of similar results to our study was added to the manuscript. Also, the possible mechanism in relation to it was explained. Please see the revised manuscript.

19.L. 326; Please, revise the italics (*Zea mays* L.)

Authors' response: We have corrected the italics as pointed out by the reviewer.

20. Table 5: Please, add the meaning of the letters accompanying Ni concentrations

Authors' response: The meaning of the letters has been added to the caption of Table 5, as suggested by the reviewer.

21.331: RH300 also statistically differs (Table 5)

Authors' response: Application of all the biochars caused a significant decrease in the Ni-shoot content compared to the C (Table 5). The sentence "**Only the RH500 and SM300 treatments differed statistically from each other**" was removed to avoid ambiguity.

22. Why SM300 statistically differs from C, but SM500 does not? (Table 5)

Authors' response: The significant letter associated to the control treatment (A) was different with the significant letters attributed to the SM500 (BC) and SM300 (C). Thus, both of them (SMB300 and SM500) had statistically different compared to control. Please see Table 5.

23.348 – 349: "Several studies have investigated the effect of soil Si application on shoot Ni concentration in various plant species" Which studies? Please, add them on the manuscript.

Authors' response: We added two studies about the effects of Si on reducing the shoot Ni and Pb concentration in two different plants. Please see the revised manuscript.

24.387: Heavy elements? Do you mean heavy metals?

Authors' response: Thank you for pointing out this error, it has been corrected to heavy metal.

25.574: Please, revise the italics (*Brassica...*), Also in L. 514, 505, 558, 514, etc. Please, revise the italics along the manuscript (particularly in the reference list)

Authors' response: We have corrected all instances of where scientific plant names were not italicized as pointed out by the reviewer.

ANONYMOUS REFEREE #2

Summary

Interesting study that could provide useful information in regard to the immobilisation of Ni in agricultural soils. However, the manuscript needs a significant re-write before it is in any way acceptable for publication.

General comments

- 1. The standard of English is generally good but improvements should be made throughout to ensure smooth reading, especially related to the use of the abbreviation PTEs.**

Authors' response: The manuscript has been thoroughly revised according to the reviewers' comments, and English improved.

- 2. There is a distinct lack of detail in all sections, all of which would benefit from considerable expansion. The reader should be able to replicate this experiment exactly in the same way the authors carried it out – impossible currently with the paucity of information.**

Authors' response: The manuscript materials and methods section has been thoroughly revised according to the reviewers' comments to improve description of methodology used. See responses to specific comments of Reviewer 1 and 2.

- 3. More thought should be given to presentation and interpretation of results and results should be better compared to available literature. Please don't split tables over 2 pages, very difficult to review.**

Authors' response: The manuscript Results and discussion section has been thoroughly revised according to the reviewers' comments to provide more specific explanations and discussions of the results in comparison to the scientific literature. See responses to specific comments of Reviewer 1 and 2. Care has been taken to ensure that tables are not split over 2 pages.

Specific comments

- 4. More background information is needed throughout the manuscript to enhance understanding of the need for the experiment and also on contamination and soil processes and how these relate to the importance and findings of this study. The Introduction needs to be greatly expanded to include more information on why this study is so important, especially for a country like Iran. For example what area of the country is available for agriculture, how much is needed/is it enough? How much is land is contaminated and with what...? Why is Ni particularly relevant?**

Authors' response: Thank you for your suggestions. We added latest information available about of Ni content in agricultural soils of Iran to the introduction section. Please see the revised manuscript.

- 5. Also, in the introduction much more detail on the various soil processes/mechanisms/chemistry related to the bioavailability of PTEs, their distribution between various soil fractions and how mechanistically additions/amendments increase or decrease both the bioavailability of PTEs like Ni in soil and the enhancement or otherwise of crop growth.**

Authors' response: We added more specific mechanistic information on how biochar reduces Ni bioavailability in soils to the Introduction section. Please see the revised manuscript.

- 6. Lots of things are stated without giving any examples. E.g. L73 "The quantity and rate of release of soil PTEs from soil particles over time can influence their bioavailability". In what way? Is it the same for all PTEs? How does Ni compare to other PTEs? Are the processes generally the same? How do they differ? Also add details on climate, topography and the variety of soil types in Iran, are the soils studied representative? Coordinates of sampled soils contaminated and otherwise. Etc. Maps would be useful – show location of sites in Iran at a minimum.**

Authors' response: Regarding PTEs release from soil, for clarity and better understanding, some changes were made in the Introduction section as suggested by the respectable reviewer. Also, a brief description of the dominant soils of Iran with their characteristics was added in Section 3.1. (Soil characteristics). As mentioned in Materials and Methods section 2.1; we collected an uncontaminated composite soil sample from research farm of the College of Agriculture and Natural Resources in Darab, southern Iran (28° 45' 0.99" N 54° 26' 52.14" E, Elevation 1105 m) and then, they were artificially contaminated by nickel. At the same time, we added the geographical coordinates of the sampling area to the manuscript. It should be noted that a comprehensive map of places contaminated with toxic elements has not yet been prepared in Iran.

Technical corrections

7. L 18 Please delete “remains” as other studies have been carried out on this topic. Or be more specific.

Authors’ response: We have rewritten the statement to be more specific and highlight the novelty of this study. It now reads: “No previous studies have examined the potentially synergistic effect of Si and biochar on soil Ni chemical fractions and immobilization.”

8. L18 Please change to “affects the immobilization of PTEs...”

Authors’ response: The sentence has been completely rewritten in response to the reviewer’s previous comment no. 8.

Hereafter read “please” for all changes

9. L22 Sort out S1 closing bracket

Authors’ response: The correction has been made as suggested by the reviewer.

10. L23 Change included to including

Authors’ response: The correction has been made as suggested by the reviewer.

11. L34 Add “of” between potential and Si

Authors’ response: The correction has been made as suggested by the reviewer.

12. L37 Add “through” between the and consumption

Authors’ response: The correction has been made as suggested by the reviewer.

13. L45 Expand on contaminated sites and what the relevance is – perhaps percentage area of the country, why it is important to use this land. What are the expensive techniques used...

Authors’ response: In this section, the latest official information reported about nickel content in Iranian soils and the percentage of nickel-contaminated soils was added. Also, the cost methods for remediating contaminated soils were briefly mentioned.

14. L51 Do you mean “soil stress caused by PTEs?”

Authors’ response: We have rewritten the sentence to improve clarity. It now reads: “Applying Si to the soil can enhance plant resistance against biological and non-biological tensions, including physiological stress caused by PTEs in soil”

15. I will stop with comments on English here – manuscript needs the English to be improved throughout, specifically I would read out the abbreviation for PTEs when rewriting – see previous comment and rearrangement of word order, e.g. L67 should read “in stabilizing PTEs in soils...”. L68 reads “Soil potentially toxic elements...”, no, “potentially toxic elements in soils...”

Authors’ response: We have corrected all instance of soil PTEs to PTEs in soils as suggested by the reviewer.

16. L53 Give example of other materials that are not as good for remediation

Authors’ response: the better sentence was replaced to clarify. Please see the revised manuscript.

17. L55 Silicon itself does not directly increase soil pH. However, silicon can indirectly influence soil pH through various mechanisms – please provide some background / further expansion on this

Authors' response: We have added a sentence to explain the soil pH increase due to Si application.

18. L71 delete “as”

Authors' response: The correction has been made as suggested by the reviewer.

19. L73 what characteristics?

Authors' response: some important soil characteristics were added. Please see the revised manuscript.

20. L74 – expand on this process – give some examples on how bioavailability can be influenced

Authors' response: Please see the author response for question 6.

21. L88 – remains – see previous comment L18, or be more specific

Authors' response: The sentences have been rewritten as follows to be more specific: “As both biochars and Si are economical and effective soil amendments to reduce plant PTE uptake and stress in contaminated soils, their potential synergistic effect on the immobilization of PTEs in soils should be further investigated. Currently, no previous studies have examined the combined application effects of Si and biochars the chemical fractions and release kinetics of Ni in calcareous soils.

22. L96 polluting? Pollution? What characteristics?

Authors' response: The section 2.1 heading has been corrected as follows: “2.1 Soil sampling, characterization and nickel treatment”.

23. L97 Section 2.1 – add more details on general soil characteristics (how do these compare for example to other Iranian soils and to European agricultural soils) laboratory methods /equipment.

Authors' response: We have added a concise description of each of the standard international methods used to determine the soil properties with references, as also suggested by Reviewer 1.

24. Also more detail on the method of Boostani et al., readers don't want to have to go searching in other papers to find out what you have done – provide brief description at a minimum. Ni(Cl₂) solution, more details on concentration, purity, supplier....

Authors' response: We have added a concise description of the Ni treatment methodology, as also suggested by Reviewer 1. Also, the characteristics of applied Ni(Cl)₂ was added.

25. How were soils sampled? Where, with what? How was representativeness ensured? L104 Section 2.2 – ditto above, much more detail required.

Authors' response: Section 2.2 was revised as suggested by the respectable reviewer.

26. Where did the feedstocks come from? What are they? What weights were used? What kind of mesh? Material? Supplier?

Authors' response: The requested information about feedstocks were added to the manuscript in the section 2.2. please see the revised manuscript.

27. L113 and throughout manuscript – space between all temperatures and °C

Authors' response: The correction has been made throughout the manuscript as pointed out by the reviewer.

28. L116 contaminated soil samples – this just appears from nowhere. What contaminated soil? From where? Contaminated with what and by how much? How was the contaminated soil sampled? How many sampling points? Were all the samples combined and then subsampled?

Authors' response: The term “contaminated” has been replaced by “Ni-treated” to correspond with Section 2.1, where the Ni treatment (contamination) of the soil is explained.

29. Section 2.3 – much more detail required – what are the pots? How many pots? What weight of soil in the pots? Controls? Replicates?... What required reactions? Why was distilled water used to keep soil at field capacity? Is field capacity representative of Iranian soils? If not how are results extrapolated to reflect average water content in soils in this part of Iran?

Authors' response: As suggested by the respectable reviewer, the type of pots, the number of pots and the amount of soil in them were added to the manuscript. Because a rich source of carbon has entered the soil, we gave it two weeks to minimize the activity of the soil microorganisms to prevent competition between the microorganisms and the plants for nitrogen absorption in the early stages of plant growth. We used distilled water to irrigate the pots during the growing season, so that no substances are added to the soil through the irrigation water and the conditions are controlled. At field capacity level, the plant uses the least amount of energy to absorb water, and if we considered a lower soil moisture content, the plant might experience moisture stress in addition to the Ni stress.

30. Section 2.4 – ditto above sections – provide enough detail that other researchers can repeat exactly what you have done. Details on chemicals, suppliers...

Authors' response: We added more detailed on the chemical used in each of the sections as suggested by the respectable reviewer. The extraction method of Singh et al. (1988) is a well-known method for soil scientists to separate the forms of heavy elements in soil, and usually it is enough to mention this method in published articles, although Table 1 In short, shows the working method and the chemicals used in it.

31. Section 2.5 Ditto above and improve English

Authors' response: We have checked the English grammar again and corrected it. we added more detailed on the chemical used in each of the sections as suggested by the respectable reviewer.

32. Section 2.6 Ditto above and improve English

Authors' response: We have checked the English grammar again and corrected it. We added more detailed on the chemical used in each of the sections as suggested by the respectable reviewer.

33. L153 Discussion not discussions

Authors' response: The correction has been made as suggested by the reviewer.

34. L155 and on, Section 3, how was SOM quantified? Ditto CEC? This is not mentioned in section 2. Ditto all other determined values listed in Table 2.

Authors' response: We have added a concise description of each of the methods used to determine the soil properties with references, as also suggested by Reviewer 1.

35. L155 What uncontaminated soil?

Authors' response: The sentence has been rewritten as follows to clarify the intended meaning: "The soil used in the study prior to experimental treatment, exhibited a sandy loam texture and possessed alkaline properties with significant calcium carbonate content, while not being classified as saline (Table 2)."

36. L165 How exactly was the pH, EC and other parameters listed in Table 3 determined?

Authors' response: We have added a concise description of each of the methods used to determine the biochar properties with references, as also suggested by Reviewer 1.

37. L171 Surface functional groups and CEC – add some information on this to the Introduction

Authors' response: A sentence has been added to the Introduction regarding biochar surface functional groups and CEC.

38. L181 Expand on how the ratios indicate what you say here

Authors' response: The sentence has been amended to explain the ratios as follows: "The ratios of H:C and O:C are significant indicators of the aromaticity and polarity of biochars; the lower the ratios the more condensed aromatic C the biochar contains (Chatterjee et al., 2020).

39. L182 The results "displayed in" Table 3. Table 3 did not by itself produce results.

Authors' response: We have corrected "of" to "shown in".

40. Where are the uncertainties in Table 3? Are differences in this table significant or not?

Authors' response: Usually, the values of the properties related to the amendments are expressed quantitatively and they are not compared statistically. Only in some cases, the standard deviation of each value is given.

41. L184 indicate not indicated

Authors' response: The correction has been made as suggested by the reviewer.

42. L185 Insignificant how? Compared to what? As it appears none was detected

Authors' response: The sentence has been rewritten as follows to improve clarity: The Ni content in the biochars derived from rice husk was below detection, whereas a limited quantity of Ni was detected in the biochars produced from sheep manure (Table 3).

43. L186 – limited yes but more than the RH biochar and is the difference between SM300 and SM500 significant?

Authors' response: Here, only more or less is considered and statistical comparison is not customary for it. Please see the author response for comment 42.

44. L188, Section 3.3 FTIR and SEM come out of nowhere. Details in the methods section please.

Authors' response: A description of the FTIR and SEM methodology used to characterize the biochars was added to Section 2.2 as also requested by Reviewer 1.

45. In this section replace evident with evidenced or rather "as indicated"

Authors' response: "evident" was replaced by "as indicated" as suggested by the reviewer.

46. L205 Figure 2

Authors' response: We have corrected the Figure number as also pointed out by Reviewer 1.

47. L252 Figure 3

Authors' response: This particular discussion regarding Ni-Car is correctly referring to Fig. 4, and so we have not changed it.

48. Figure 3 – why sum Si and biochar? Why not show them separately?

Author's response: Figure 3 illustrates that the synergistic effect of the combined treatment is substantially greater than simply the sum of the two treatments separately.

49. L 213 Change to "Soil Ni chemical fractions after addition of silicon and biochar"

Author's response: The section 3.4 heading has been amended as suggested by the reviewer.

50. L215 – significant how? What happened to the fractions? Confusing, please re-write.

Author's response: The sentence has been rewritten to improve clarity. It now reads: "The main effects of treatments (biochars and Si levels) and their interactions had a statistically significant effect ($P < 0.01$) on all the soil Ni chemical fractions, except for the Ni-Car fraction, where only the main effects were significant".

51. Actually many paragraphs in this section (3.4) need to be revised as there are a lot of random observations: Table 4 results are discussed, then Table 3, then Figure 1 without a logical coherent story/interpretation. Again, a better review of the literature in the introduction would help here, i.e. what previous studies have shown when it comes to amendments to soils, and their affect on PTEs and crop production.

Author's response: Section 3.4 discussions have been revised according to the reviewer's suggestions. Also, we tried as much as possible to add content that would contribute to the richness of the manuscript as long as the introduction section is not too long. Please see the introduction section in revised manuscript.

52. L239 Was microbial activity measured? Can you really say with certainty from lower H/C mole ratios that there were lower rates of microbial oxidation?

Author's response: In this experiment, we did not measure microbial activity, and based on the results of Sun et al. (2023) as given in the text of the manuscript, we expressed this mechanism as a possibility.

53. Figure 4 – Ni not Cd on y axis

Authors' response: The y-axis has been corrected as also pointed out by Reviewer 1.

54. Table 4 What are the values in bold in the right-hand column?

Authors' response: We have added the word "Mean" to the heading of the right-hand column in Table 4.

55. L252 State from what concentration to what concentration that Ni decreased by (11.7%).

Authors' response: the Ni concentrations in the CAR form associated with the S₀ and S₂ treatments were indicated as suggested by the respectable reviewer.

56. Is Ni-Car or Car-Ni? Figure 4 Cd-Car

Authors' response: The axis has been corrected to Car-Ni.

57. L275 rates?

Authors' response: In agronomy and soil science, an application rate refers to the mass of amendment applied per mass or area of soil.

58. L276 Define BCR

Authors' response: BCR has been defined in the text.

59. L278 Application rate? A rate implies speed of application

Authors' response: In agronomy and soil science, an application rate refers to the mass of amendment applied per mass or area of soil.

60. L282 Why the difference between SM biochar and the others? Provide some more detail, mechanisms

Authors' response: Regarding the change in the amount of nickel bound to organic materials under the influence of biochars, an extensive comparison was made with the literature. But in general, the mechanisms related to it are not completely clear yet and conflicting results have been reported in different studies. Please see the revised manuscript.

61. L291 - L292 rates? The addition of S0 is not an addition – nothing was added – please rephrase

Authors' response: The sentence has been rewritten to improve the wording. It now reads: "Furthermore, increasing the Si concentration from S₀ to S₂ significantly decreased MnOx-Ni by 17.6% (Table 4)."

62. L294 C+S0 is not a treatment -it is a control with no addition – please rephrase

Authors' response: The sentence has been rewritten to improve the wording. It now reads: "The interaction effect of treatments showed that the highest and the lowest MnOx-Ni concentrations were found in the untreated control (11.58 mg Ni kg⁻¹ soil) and combined SM300+S₂ (2.98 mg Ni kg⁻¹ soil), respectively (Table 4)."

63. Table 7 - mg Cd kg-1 h-1??

Authors' response: We have corrected the unit in Table 7 to Ni as pointed out by the reviewer.

Without going further this manuscript need a significant re-write before it is in any way acceptable for publication. If the authors revise the manuscript taking into account the above comments and apply the same thoroughness to the remainder of the manuscript I will happily review this again from the beginning.