

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

The manuscript has greatly improved in quality, thank you for accepting the previous suggestions. However, I just have one small issue:

1. **In L. 131 - 136, indeed the methodology of soil incubation with Ni has been developed a little more. However, the incubation conditions: temperature, darkness, duration have not been included. This information must be added.**
In addition, I would like a short explanation on how the drying and rewetting cycles contribute to equilibrate Ni with the soil.

Authors' response: some available information about incubation conditions were added as suggested by the respectable reviewer. Please see the revised manuscript. we developed the drying and rewetting cycles for soil contamination with Ni to simulate real field conditions.

2. **L. 145: "Biochar pH and EC were determined...". Same comment in L. 147 ("Biochar total C, N and H contents were determined...")**

Authors' response: these corrections were done as suggested by the respectable reviewer. Please see the revised manuscript.

3. **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. Reviewer response: OK, I understand that it may not be possible to measure the amount of SOM, but then the original sentence "It has been shown that the application of Si to cultivated soils resulted in a reduction of soil organic matter content" should be written in a hypothetical way.**

Authors' response: The sentence given in this section about the reduction of the amount of soil organic matter, as influenced by Si application, is the result of the others (Ma et al. 2021) to confirm the reduction of the amount of Ni in the OM fraction in the present study. The sentence was revised in such a way that the doubts of two referees were completely resolved. Please see the revised manuscript.

ANONYMOUS REFEREE #2

Summary

I acknowledge the efforts the authors have made to revise and improve the manuscript, for example section 2 is much better. However, significant work is still needed before the manuscript is acceptable for publication. For example in the introduction more information is required in order to provide better context and the relevance/importance of the study. I would appreciate if after making all the corrections

that the authors read through the manuscript again carefully, at least twice, as it seems to me, that in a rush to get it re-submitted a plethora of careless mistakes have been made/missed.

Corrections

1. **Abstract: there might be no previous study like this one, but why then is it important/relevant? Please say why reducing the bioavailability of Ni is important. How much agricultural soil is contaminated with Ni? The first sentence should start with “In Iran, X% or a significant percentage of agricultural soils are contaminated with a range of PTEs including Ni, with levels ranging from X to X···.” Then Si is a**

Authors’ response: thanks for the valuable suggestion. We added a brief sentence at the beginning of the abstract to satisfy the opinion of the respectable reviewer. Please see the revised manuscript.

2. **L19 interactive effects, delete levels,**

Authors’ response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

3. **L20 biochar singular, replace alleviate with “reduce”**

Authors’ response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

4. **L38 replace using with “use of”**

Authors’ response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

5. **L44 replace “it” with Ni**

Authors’ response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

6. **L46-54 The information on the Shabhazi study should come first to provide the context. In a study conducted by Shabazi et al....**

Collected soils from different climates? What does this mean? Climates don’t have soils. Climate is typically defined as the long-term patterns of temperature, humidity, wind, precipitation, and other atmospheric conditions in a particular region. Climatic regions? As mentioned in the last review, more background is required. For example something like the following but with references and put more succinctly:

Land type

"Iran is a country known for its diverse geography, ranging from deserts to mountainous regions, which affects the availability of land suitable for agriculture. Arable Land: Arable land refers to land suitable for cultivation of crops. In Iran, arable land accounts for approximately 10% to 12% of the total land area. Cultivable Land: This category includes not only arable land but also land suitable for other forms of agriculture such as orchards, vineyards, and pastures. The percentage of cultivable land in Iran is slightly higher than arable land, estimated to be around 16% to 18% of the total land area. Irrigated Land: Iran has a significant reliance on irrigation for agriculture, particularly in arid and semi-arid regions. Irrigated land accounts for approximately 10% to 12% of the total land area. Marginal Land: Some portions of Iran's land are considered marginal for agriculture due to factors such as soil quality, topography, and water availability. The extent of marginal land varies across regions but can be significant, particularly in arid and mountainous areas. Overall, while Iran has considerable agricultural potential, it also faces challenges such as

water scarcity, soil degradation, and limited access to modern farming technologies, which can affect the actual percentage of land available for agriculture and agricultural productivity.”

Climate – use the Köppen classification

BWh: Hot Desert Climate (Arid):

BSk: Cold Semi-Arid Climate:

Csa: Hot-Summer Mediterranean Climate:

Csb: Warm-Summer Mediterranean Climate:

Dsa: Cold Semi-Arid Climate:

ET: Tundra Climate:

☑ Characterise the climate, average annual precipitation etc. of your study area

☑ Please add a summary of the background above before introducing the Shabazi Study

All the above information should provide good background to the study being carried out and underscore the importance/relevance of the study.

Authors' response: according to the reviewer suggestion, we changed the sentence to “**In a study conducted by Shahbazi et al. (2022), the Ni weighted average concentration of the cultivated lands of Iran in the vicinity of the industrial areas was reported 349.8 mg kg⁻¹ soil**” for more clarity. Furthermore, we complete the sentence of “Shahbazi et al. (2020) collected.....” based on the referee suggestion and changed it as follows to more clarity: **Shahbazi et al. (2020) collected 711 agricultural soil samples located at different climate zones (extra arid, arid, semi-arid, Mediterranean, semi humid, humid and per-humid based on the de Martonne classification system) of Iran.** As you know, the climate is one of the most important soil-forming factors and the properties of soils at different climate zones are totally different. We also added the climate, mean annual precipitation, soil moisture regime and soil thermal regime of the study area in the materials and methods section (soil sampling). Please see the revised manuscript.

7. L47 2 significant figures is enough - 350

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

8. L61 mitigate “the toxicity of PTEs....

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

9. L63 reduce “the bioavailability of PTEs...

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

10. L64 and “the formation of...

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

11. L68 Biochar can be used for many things including water filtration. Change to “Biochar can be used for a number of applications including... .

Authors' response: There is no such sentence in the text of our manuscript.

12. L81 Increased, L86 organic matter, L89 PTEs don't have chemical fractions. Rephrase, PTEs in other...., L100 the bonding of...

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

13. L105 Rewrite the latter half of this sentence after oxides

Authors' response: we have rewritten it as follows: while the residual and organic matter-bound forms experienced a notable enhancement. Please see the revised manuscript.

14. L107 Explain what light texture means

Authors' response: we have added soil textural class of the soil for more clarity. Please see the revised manuscript.

15. L111 “..reduce plant PTE (potentially toxic element) uptake... change to plant uptake of PTEs. Again, this PTE word order issues was mentioned previously. Read out loud all the abbreviation in the text in full to ensure correct word order.

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

16. L116 change alleviate to reduce

Authors' response: We have made the corrections within the manuscript as suggested by the respectable reviewer. Please see the revised manuscript.

17. But for section 2.1 I still strongly suggest adding a map of Iran or Southern Iran to show arable regions, where your study area is and how close it is to industrial areas – see below paper as an example

Authors' response: We have added a map to the manuscript (2.1) which shows the exact location of soil sampling. Please see the revised manuscript.

18. L121 with an auger and placed into what? How was the sample then transported to the lab. How were they stored in the lab before preparation?

Authors' response: After collecting the soil samples from the field by auger, they were mixed completely and after air drying and passing through a 2 mm sieve, the soil sample was placed in a plastic bag and transported to the laboratory. In the laboratory, it was kept in a dark room until treating.

19. L124 Sieve material?

Authors' response: thanks for your valuable comment. For better understanding, sieve word replaced with pass. Please see the revised manuscript.

20. L152 determined

Authors' response: we have corrected it. Please see the revised manuscript.

21. L173 “from” the pots. In what and where was the soil stored before analysis?

Authors' response: we have corrected it. Also, please see the response number 18.

22. L183 millilitre should be singular here. In addition please standardise, millilitre, ml or mL (L 131)?

Authors' response: we have corrected them. Please see the revised manuscript.

23. L195 space between brackets, L197 “between” the individual...

Authors’ response: we have corrected them. Please see the revised manuscript.

24. L214 Low relative to what? Remind the reader that the average for Iran is 350 mg kg⁻¹. Why did you use a soil with such a low Ni content for this experiment?

Authors’ response: Here, the respected referee should be reminded that according to the study of Shahbazi et al. (2020), the average Ni in the soils of different regions of Iran is 68 mg kg⁻¹ of soil. The average of 350 mg kg⁻¹ is related to the soils in the vicinity of industrial areas (Shahbazi et al. 2022) (see the introduction section). In general, according to Iran's environmental standard, if the soil contains more than 100 mg Ni kg⁻¹ soil, it is considered contaminated. For this reason, we chose the 300 mg Ni kg⁻¹ soil for soil contaminating in this study.

25. L236 indicate, L242 3.3 Change to “Biochar analysis using FTIR and SEM”, L255 FTIR “spectra”... delete “of”, L264 SEM “images”,

Authors’ response: we have corrected them. Please see the revised manuscript.

26. L269-271 You say effect of treatments were significant except for NI-Car fraction, and then you say “where only the main effects were significant”. Confusing – please re-write this sentence.

Authors’ response: thanks for your valuable comment. We have rewritten the sentence for better understanding. please see the revised manuscript.

27. L271-272 for which biochar is this? State the concentrations in addition to the % reduction.

Authors’ response: Here, we have discussed on the main effects of Si levels on reduction of WsEx fraction. We also added the concentrations. Please see the revised manuscript.

28. L274 As above, state the concentrations – from xx mkg-1 to xx mg kg-1

Authors’ response: We have added the concentration changes. Please see the revised manuscript.

29. L281 For clarity state what is being reduced in the WsEx fraction

Authors’ response: for better clarity, we have added Ni to WsEx (Ni-WsEx). Please see the revised manuscript.

30. L295 delete probably, L296 replace “indicated” with “reflected”, replace “by” with “in”, replace “higher” with “greater”, L297 replace “reflected” by “indicated”. Add “the” between by and lower

Authors’ response: we have corrected them. Please see the revised manuscript.

31. L304 Sentence beginning “Due to the...” How does this information from the Ma et al., study relate to this study?

Authors’ response: In this research, the source of Si was sodium metasilicate. Because the application of Si levels in the present study increased soil pH, the presence of this sentence is to confirm the content and how sodium metasilicate increases soil pH.

32. L314 Change to “Si application rates from..., L315 2 at most 3 sig figures is enough. The same goes for Table 4 and Table 3, L318 “mine contaminated soil”? ...a soil contaminated by mining activities..., L322 PTEs and word order again. ...bioavailability of PTEs in soil...,

Authors' response: we have corrected them. Please see the revised manuscript.

33. L321 State what this experiment used as an amendment

Authors' response: the type of amendment material was added. Please see the revised manuscript.

34. L324 “The decrease in the concentration of Ni in the carbonate form with an increase in the Si levels...”, to what is this referring? As found in this study? What treatment/s?

Authors' response: thanks for your valuable suggestion. we moved this sentence to the desired location. Please see the revised manuscript.

35. L337 “The greatest OM-Ni reduction (18.6%) was due to SM500”. Rephrase – see line 314 “... the greatest reduction in soil Ni content in the OM fraction was found to be in that which underwent the SM500 treatment...”

Authors' response: we have corrected the sentence as suggested by the respectable reviewer. Please see the revised manuscript.

36. L341 Relace “they” with the study,

Authors' response: It seems that it is more correct to use the word of “they” here.

37. L342 delete “as affected”, L344 “...in the organic matter cadmium fraction...” sounds strange, change to “... Increase in Cd in the OM fraction...”

Authors' response: we have corrected them. Please see the revised manuscript.

38. L348, L349 – see L344, L349 – no fighting going on as far as I am aware. Please replace “conflict” with “contrast”, L350 Change “According to the above-mentioned points, it seems that..” to “These observations point to the fact that... or “These observations indicate..., L354 – see L344,

Authors' response: we have corrected them. Please see the revised manuscript.

39. L353-354 For which treatment? Or is this a mean? Please add this info.

Authors' response: in this sentence, the treatment is clear. By application the Si levels.....

40. Table 4 – where is the Ni mobility factor (%) referred to in the table caption and notes? Is this whole table not concentrations of Ni in mg kg-1?

Authors' response: thanks for your accuracy. We have deleted the Ni mobility factor from the topic of Table 4. Please see the revised manuscript.

41. L354-355 Where has it been shown? References.

Authors' response: The sentence was revised in such a way that the doubts created for both referees in this part are resolved. Please see the revised manuscripts.

42. L356 Start with “In this study, the interaction...., L357 – see L344, L359 C+S0 is not a treatment it is a control – please rephrase, L360 – see L344, L361 “the” control. Delete “was”. Replace “attributed to” with “caused by”. “.. the SM300 treatment” . Replace “by” with “at”

Authors' response: we have corrected them. Please see the revised manuscript.

43. L363 – see L344, L364 .. at “a” lower..., L366 .. at “a” higher..., L367 Delete “the” after increasing,, L368 Replace “low” with lower, temperature with temperatures,,
Authors’ response: we have corrected them. Please see the revised manuscript.

44. L367-L369 Where does this information come from? Provide reference or start the sentence with “It is well known....

Authors’ response: thanks for your valuable suggestion. we have added the Phrase of “It is well known... In the beginning of sentence. Please see the revised manuscript.

45. L371 Change “...the exchangeable and water-soluble Mn concentration...” to “.. the Mn concentration in...” L373 – see L344, L373-375 The control is not a treatment. Start sentence with “Compared to the control which had the highest concentration of Ni in the MnOx fraction, the greatest interactive effect was.... L376 concentration not concentrations “..the” AFeOx and CFeOx “fractions was...” L382 – see L344,

Authors’ response: we have corrected them. Please see the revised manuscript.

46. L384, delete “the” after however. Only the “mean value” of the control and the 2 different applications of Si for the RH500 treatment...

Authors’ response: Here, there is no discussion of silicon application levels. Here, the main effects of biochars application on changes the concentration of soil Ni in the form of AFeOx is given.

47. L385 Delete B from SMB

Authors’ response: we have corrected it. Please see the revised manuscript.

48. L386-387 – what do you mean by this? By form do you mean “fractions”. It seems to me that the addition of Si did affect the Ni concentration in this fraction. E.g. for SM 200, S1 and S2 significantly decreased the concentration of Ni compared to the control. There are other examples for this fraction.

Authors’ response: Here, the main effects of the Si levels on the changes of the soil Ni Res fraction is considered. The sentence was modified for better clarity. Please see the revised manuscript.

49. L388 In this study was contaminated or amended soil used?

Authors’ response: as clearly described in the Materials and Methods section, in this study, we contaminated the calcareous soil with Ni in the level of 300 mg Ni kg⁻¹ soil (it is marked with “Ni-contaminated soil” within the text of the manuscript) and then, we apply the Si and biochar as amendment materials for Ni immobilizing process.

50. L390 Ni in the Res fraction and reduced Ni in the other fractions

Authors’ response: we have corrected it. Please see the revised manuscript.

51. L392-393 Did the amount of the fractions increase or did the Ni increase in the fractions? Rephrase entire sentence. L394-396 Ditto

Authors' response: we have corrected the sentence in order to more clarify as suggested by the respectable reviewer. Please see the revised manuscript.

52. L397 PTE again, rephrase, L401 Ni did not transform, it moved or transferred more into other fractions,

Authors' response: we have corrected it. We replaced the word of "transformation" by "transfer". Please see the revised manuscript.

53. L404 replace "use" with "application", L405 replace was with "is"

Authors' response: we have corrected them. Please see the revised manuscript.

54. L403-405 The RH500 with Si was also effective for this fraction

Authors' response: its true. But for the SM treatments were more effective than others. We have added the word of "more" before "evident" in the sentence for better clarity.

55. L407 "application" rates, replace "interactions" with interactive effects

Authors' response: we have corrected them. Please see the revised manuscript.

56. Table 4 and Table 5 captions and text – standardize the addition of Si. You have Si application levels (Tab 5), silicon levels (Tab 4) and "application rates" or just "Si rates" (L407) in the text

Authors' response: as suggested by the respectable reviewer, we standardized it as Si application levels. Please see the revised manuscript.

57. L407-408 Change to "With the exception of SM500 (S0) use of biochar, Si application and their interactive effects were all had a statistically significant effect on shoot Ni concentration."

Authors' response: Here, it is meant to express the significance of the main effects of biochar treatments and Si levels and their interactions on the Ni shoot content base on the ANOVA.

58. Table 5 (and actually also Table 4): I am not sure of the value of calculating the mean of different treatments. Surely the point is to compare the effects and interactions of the individual treatments? What does taking the mean of the control with no silicon, the control with 250 mg kg Si and the control with 500 mg kg Si bring? Likewise what is the point of taking the mean value of the control with no silicon and 4 different biochars with no silicon bring? The data of real value in the table are which silicon application level and which biochar reduce the amount of shoot Ni – i.e. SM500 and SM300 S2.

Authors' response: When the data are analyzed by statistical software, the significance of the main effects of the treatments (levels of Si and biochars each alone) and their interactions are determined. The purpose of expressing the main effects of treatments, for example, the effect of biochar application, is to determine the best biochar treatment, for instance, in reducing the Ni concentration of shoots, without considering the effect of Si application. The purpose of expressing interactive effects is to compare the effect of all the combined treatments of biochar and Si (15 treatments) and determine the best combined treatment, for example, in reducing the concentration of Ni in the shoot.

59. L408-409 32%? For which treatment? Seems to me that SM500 S2 has more than 50% less Ni relative to the control with no silicon and about 50% of the shoot Ni relative to the same biochar with no Silicon. L409-411 – SM500 (S0) does not have a significantly different shoot Ni concentration compared to the control

Authors' response: please see the above response. we first indicated the main effects of Si levels and biochar, separately and in following, the interaction effects are given. 32% decrease in shoot Ni concentration is associated with the main effects of Si application levels.

60. L411 Replace interaction with interactive, L412 C+S0 is not a treatment, it is the control by which all treatments are compared. Rephrase,

Authors' response: we have corrected them. Please see the revised manuscript.

61. L413-416 – there is no data shown to confirm this statement and this sentence is a bit random. Perhaps start with something along the lines of that you compared shoot Ni concentrations with Ni concentrations in the soil fractions. From this analysis it was found.... Similarly soil pH.

Authors' response: In this manuscript, we have presented all the data related to the chemical fractions of Ni in the soil, the concentration of Ni in the plant and the soil pH under the influence of the application of Si and biochar levels. In this part, we have brought the results of Pearson correlation between these parameters (correlation coefficient and probability level) which has been done by SPSS software and conclusions have been introduced according to it. It seems that, the content is very well-founded and logical.

62. L419 Change “reduction of shoot Ni concentration of spinach...” to “a reduction in the concentration of Ni in spinach shoots...L420 Add “of” between application and rice. Delete 2nd application. Was the reduction significant? Say so either way. L423 PTE – rephrase

Authors' response: we have corrected them. Please see the revised manuscript.

63. L424 What do you mean by “surface adsorption” is a significant factor? Rephrase. Surface adsorption by what? L425 What do you mean by “altered redox conditions of PTEs”, be more specific. L433 Maize not maze, Pb concentration in shoots not Pb-shoot concentration. What are lead-shoots? L434 Ni concentration in shoots, L438 see L434

Authors' response: we have corrected them. Please see the revised manuscript.

64. L447 Fig. or Figure – standardize throughout according to journal format

Authors' response: When this word is used in the text, it is written in full, and when it is in parentheses, it is abbreviated. Abbreviations are also given for figure captions. Please see the revised manuscript.

65. Fig. 5 y-axis caption – capital C, y-axis - no need for .00, 2 sig. figures enough. The colour coding for the different treatments could be improved as there is not much difference between the colours of some of the treatments. At least make the control red or some stronger colour to stand out, maybe start the axis at 5 to spread the different points out a bit more.

Authors' response: we have done the corrections for the Figure 6 as suggested by the respectable reviewer. Please see the revised manuscript.

66. L453 Add “the” between higher and pyrolysis, delete “the” between reducing and soil. L454 “the” lower... L455 C+S0 – again, not a treatment – this is the control with nothing added, L464 kinetic,

Authors’ response: we have corrected them. Please see the revised manuscript.

67. L490 did you contaminate the soil or amend it?

Authors’ response: please refer to the response number 49.

68. L492 kinetic, L493 metal, L494 “the” treatments. Replace “of all the biochar treatments” with “all 4 biochars” , L497 delete “has”, L498 see L490, L499 interactive, indicate,

Authors’ response: we have corrected them. Please see the revised manuscript.

69. L510 It looks more like 50% to me – please check

Authors’ response: it was checked again. It was true.

70. Table 7 caption “the” power... “Ni-polluted”? See L490, L522 see L490

Authors’ response: the correction was done. Also, please refer to the response number 49.

71. L541 enhances,

Authors’ response: the correction was done. Please see the revised manuscript.

72. L543 distribution of Ni between the various soil chemical forms

Authors’ response: This phrase (soil chemical forms) has no correct meaning. We changed to “the distribution of Ni chemical fractions in soil” for better clarity. Please see the revised manuscript.

73. Conclusions: Please say something about how you think your results arising from a soil amended with Ni compares to an aged Ni-contaminated soil. In other words how representative is your experiment to the real life situation?

Authors’ response: In this experiment, we have not worked on aged Ni- contaminated soils and here, we can only add it as a suggestion for future experiments. Please see the revised manuscript.