Manuscript Details

Manuscript number	GEODER_2020_841_R1
Title	Effect of soil properties on time-dependent fixation (ageing) of selenate
Article type	Research Paper

Abstract

Previous biofortification studies have established that the residual effect of added selenium (Se) fertilisers on second-season crops is minimal. To explore the fate of exogenous Se in soil, chemical and biological methods were employed to assess the change in Se bioavailability with time. Eight soils varying in physicochemical properties were spiked with sodium selenate (0.5 mg kg-1 Se) and incubated at 25°C for different periods (1, 30, 60, 90 and 300 d). At the end of the incubation, soil Se was fractionated by a sequential extraction procedure into ‘soluble’, ‘adsorbed’ and ‘organically-bound’ Se fractions. Simultaneously, wheat was grown in the Se-aged soils, under controlled conditions for six weeks, and uptake was determined. A general decrease in Se solubility over time was observed, but the rate of decrease varied depending on soil type. A reversible first order model fitted the Se ageing kinetics well, except in an Oxisol. The most pronounced ageing was observed in calcareous soils. Concentrations of Se in the shoots of wheat grown in freshly spiked soils ranged from 71.8 ± 17.5 mg kg-1 in calcareous soils to 110 ± 31.6 mg kg-1 in non-calcareous, low-OM soils. With ageing, shoot Se concentrations decreased to < 10 mg kg-1, with the lowest concentrations (0.31 ± 0.03 mg kg-1) measured in plants grown in calcareous soils. The Se concentrations in plants correlated well with the soluble Se fraction (extracted with 0.01 M CaCl2). The findings of this study can be used to make more informed decisions about the rate and frequency of Se fertiliser application in agronomic biofortification programs.

Keywords	selenate; ageing; chemical extractions; pot trial; modelling
Taxonomy	Biogeochemistry, Fertilizer, Micronutrient, Analytical Chemistry, Agricultural Soil
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Data will be made available on request

The Associate Editor
Geoderma

5th September 2020

Dear Sir/Madam,

Following an Editorial request for moderate revisions, I am pleased to re-submit our manuscript titled ‘Effect of soil properties on time-dependent fixation (ageing) of selenate’ for publication in Geoderma**.**

We have, to the best of our ability, addressed all the comments and suggestions made by the reviewers and the Assoc. Editor on our manuscript. Upon the reviewer’s request, the findings from several previous ageing studies were incorporated into the Introduction and care was taken to highlight the gap in the literature that our study addressed. The novelty of our study lies within the wide range of soils used and the combinative approach of chemical, biological and modelling techniques to test for and predict Se bioavailability with ageing time. We believe that this study provided valuable insight about the fate of added Se in various soils over time, which would be of crucial importance in ascertaining the rate and frequency of Se fertiliser application timing in future biofortification programs in different soil and climatic regimes.

We also re-run the statistical analyses on our data set, following the Assoc. Editor’s valuable advice on how to improve the robustness of the experimental data. The effect of time on the solubility of Se in the different soils was verified by a repeated measures ANOVA analysis. We assessed multicollinearity in our data set by verifying the VIF during the multiple linear regression analysis undertaken to determine which soil properties mainly influenced Se ageing. With these revisions, our experimental and modelled data analyses have been made more robust and could significantly contribute to our understanding of exogenous selenate in various soil types.

Thanking you for your time and consideration,

Sincerely,

Dr Chandnee Ramkissoon

Ref: GEODER_2020_841_R1
Title: Effect of soil properties on time-dependent fixation (ageing) of selenate
Journal: Geoderma

Associate Editor

This work examines the effect of soil properties on ageing of selenate added to eight different soils. The work has been reviewed by two reviewers. Both have found merit in their work but their recommendations are different. One reviewer recommends minor revisions, while the other recommends rejection. Thus, I am returning the work for moderate revisions. Please ensure you attend to all reviewer (and editor) comments. In particular, one reviewer raised concerns over the novelty of the work, and this needs to be addressed in any response that is supplied.

We thank the Associate Editor for his recommendation and comments. We have, to the best of our ability, made the necessary changes to the manuscript including justifying the novelty of our work.

In addition to the above, please also address the following:

There is not enough detail in the statistics section. Within the statistics section there is no mention of how the time effect is incorporated into the anova – time series work at least needs some examination of serial correlation or use of repeated measures analysis. There is also no mention of how collinearity and selection of variables was undertaken in the multiple regression, such as VIF or AIC, and the explanation of using Pearson's correlation coefficients to calculate the percentage of Se variation explained by different factors does not account for non-orthogonality of the variables or other issues well known in multiple regression (it may be appropriate if the generalised form of the equation is used, and if the above issues of variable selection are explained). There are also statements regarding 'goodness of fit' within the results which is not clearly explained in the statistics section (move this from section 3.6, as it seems to pertain to all models?).

We thank the Associate Editor for his valuable advice on how to improve the robustness of the experimental data.

Additional details have now been added to the Statistics section in the Materials and Methods to explain the different analyses used in this study (lines 236-249). Moreover, the effect of time on the change in Se bioavailability was verified through a repeated measures ANOVA analysis, which has been added to the Statistics section.

A multiple linear regression was undertaken using soil characteristics described in Table 1 as independent variables and the fraction of soluble Se at equilibrium (f_{eq}) as dependent variable. A collinearity diagnostics was run on SPSS to ensure that the independent variables were not highly correlated to each other. With a VIF of < 1.5 for all variables (except TOC and Fe, which has VIF < 2.0), it was determined that the variables were not highly correlated and our data was not showing multicollinearity. This information has not been added in the Materials and Methods (lines 243-244).

The section explaining the ‘goodness-of-fit’ has been re-written to clarify the basis on which the models were chosen to represent the ageing data. Upon the editor’s request, some of the information has also been moved to Section 3.6 (lines 229-232).

Similarly, it is not clear why or how fitting data to kinetic equations in excel can provide any robust assessment of which models best explain the data. This needs elaborating, as using solver does not prevent overfitting.

Solver is the tool we used for the modelling, but the method was least-square regression, which is the method most commonly used to find the best fit; this would also have been the method used in other software. Many scientific papers have used the Solver tool to carry out non-linear least square regression (Buekers et al., 2008; Tye et al., 2004).

The equations we used are standard equations to fit kinetic data, and have few parameters compared to the number of data points. Moreover, overfitting is mostly a concern when no theory is available to guide the modelling, which was not the case in our study. The equation that best fitted our data has already been used in several studies which describe ageing in soils (Li et al., 2016; Wang et al., 2017). Furthermore, the modelling allows us to summarize the observed trends over time in two parameters (response time and equilibrium fraction), but it does not lead to any conclusions or inferences that cannot be derived from the data themselves.

Figure 2 requires error bars.

Error bars have now been added to Fig. 2.

Reviewer #1

The bioavailability of selenium in soil is important either for biofortification and risk assessment when selenium added in soil. Usually we use selenium fractions to indicate the bioavailability. The article entitled " Effect of soil properties on time-dependent fixation (ageing) of selenate" by Chandnee Ramkissoon was studded on such kind issue. In general, the topic seems to be better suited for Geoderma. Manuscript contains some original scientific information and results. Materials and methods are well described. The use of methods is appropriate but nothing is new. However, some works have already been reported on the processes governing the ageing of Se in the soils and relationship with soil properties. Considering on this aspect, this topic isn't new. In addition, some issues need to be clarified. Thus, I suggested the manuscript couldn't be acceptable for publish since the novelty is shortage.

We thank the reviewer for his insightful comments. We agree that the topic of our research is well suited for Geoderma, and we would hope for it to published pending the proposed revisions.

We agree that some work has already been done in regards to the ageing of Se in soils over time. However, few studies have used crop trials on the aged soils to validate the chemical methods and the models generated. Wang et al. (2017) measured Se ageing in 15 Chinese soils over one year using sequential extraction, but did not include plant measurements. Peng et al (2019) carried out a pot experiment with pak choi, but their study only included two soils and hence did not allow relating Se ageing to soil characteristics. Our study addressed this gap by combining chemical, biological and predictive modelling techniques to gain a holistic understanding of the fate of added selenate fertiliser in eight soils with contrasting soil properties over a reasonably long period of time (300 d). Using the Se concentrations of the plants at the beginning and end of the incubation period, a biological ageing factor was derived. Care has been taken to include the relevant findings while still pointing out the novelty of this particular research (lines 112-129).

It is also noteworthy that Wang et al. (2017) used the Elovich equation to describe the kinetics. The Elovich equation has mostly been used to describe adsorption, not ageing. In the cited studies, the fitted value for the "b" parameter in the Elovich equation was negative, resulting in predicted negative values at long aging times, i.e. the equation is purely descriptive in this case. In contrast, the reverse first-order equation we use has a mechanistic basis and predicts realistic soluble pools at equilibrium (=long ageing time).

Highlights

The point 1 is well known fact.

We agree that Point 1 has been observed in other published papers, hence has been removed from the Highlights.

Point 3 was also found in the previous published paper.

Alternative findings were actually observed in previous published papers. For example, Li et al. (2016) observed that the change in the available Se fraction in soil with time was best represented by a second-order equation while Wang et al. (2017) showed that the empirical model, Elovich, best represented the changes in available Se with time. Hence, the fit of a reverse first-order equation, which previously has been used to describe ageing of metal(loid)s to predict temporal changes in Se bioavailability was novel and significant for this study. No changes were made to this point in the Highlights.

Point 2 “Ageing of Se was more pronounced in calcareous soils than non-calcareous ones” is questionable since adsorption will be much bigger in the acidic soil than that in the basic soil so did for ageing. Since selenate is anion, which will compete with OH for adsorption site. Thus, the availability will be bigger at basic condition than that at acidic condition.

It is true that availability of Se in basic soils was higher than acidic ones at the beginning of the ageing period (t = 0 d) (Fig. 1). However, as with sulfate, the immediate sorption of selenate is weak, even in acid soils. The ageing over time was most pronounced in the calcareous soils. Thus, by the end of the experiment (t = 300 d), the lowest concentrations of soluble Se in the soil and the lowest plant Se concentrations were recorded in calcareous soils. This strong ageing was not seen in non-calcareous alkaline soils, indicating that the ageing was likely related to Se fixation on calcite surfaces. We believe that this finding was significant and verified by the different methods employed in the study (chemical and biological). Hence no changes were made to the text.

Thus, not so many findings were included in this manuscript.

We believe that the study actually offered a clear and concise understanding of the fate of selenate in various soils over an ageing period of 300 d. The novelty of the study lies within the wide range of soils used and the combinative approach to test for and predict Se bioavailability. It was rather surprising to observe that Se ageing was more pronounced in calcareous soils compared to soils with high organic matter (OM), suggesting that the mechanism of Se sorption onto calcite was stronger than that on OM. In contrast, most previous Se ageing studies reported soil characteristics such as Fe/Mn oxides and OM predominantly influencing selenate (Wang et al., 2017) and selenite (Li et al., 2016) ageing. Moreover, using the Se concentrations of plants grown in ‘fresh’ and ‘aged’ soils, a biological ageing factor was derived, which gave a realistic picture of the fate of selenate in soils with time.

Abstract

1. This study couldn’t give the answer for “processes governing the ageing of Se in soil”. We agree this was poorly worded. The experimental methods employed in this study allowed us to determine the factors driving Se ageing in the different soils, but did not reveal the actual processes. The text has now been re-written to clarify the aim of the study (lines 14-16).

2. Only 8 soils were used in this study, how can you ensure the big difference on physicochemical properties?
   Due to practical limits on the size of the experiment, the number of soils included in the experiment was restricted to eight. Including more soils would have meant limiting the number of replicates or sampling times in order to achieve reproducible results within a reasonable time frame. Nevertheless, the eight soils were carefully chosen to represent a wide range of physical and chemical characteristics that could potentially affect Se solubility, for example, soil pH, clay and OM contents as well as Fe/Mn oxides (Table 1), i.e. the soils do cover big differences in physicochemical properties. Hence, no changes were made to the text.

3. What are the main findings for the study should be provide at the end of the abstract.
   The main findings of the study were included in the text and broader implications of the findings have now been added (lines 29-30).

Introduction

4. Line 39-40. Not only the transport of assed selenium in soil-plant, but also the transformation processes should be considered.
   To precisely assess how Se transforms in soils, the microbial communities driving Se cycling (Fellowes et al., 2013) and speciation analysis of Se in the different fractions would also need to be explored and since this was not the focus of the study, such information was not explicitly mentioned in the text. No changes were made.

   Line 62-63, "the adsorption of selenite on 1:1 aluminosilicate minerals such as kaolinite is greater than on 2:1 minerals"--- is that true? why? Please check it.
   We thank the reviewer for identifying this mistake. The adsorption of selenite is reportedly greater on minerals with greater surface area such as montmorillonite (2:1) compared to 1:1 minerals such as kaolinite. This has now been corrected in the text (lines 66-67).

5. Line 106-107, "no other studies have investigated the factors controlling ageing of Se, especially when SeVI"--- Please see the reference "Dan Wang，et al. Selenate redistribution during aging in different Chinese soils and the dominant influential factors. *Chemosphere*. 2017, 182:284-292.", This topic was studied using 15 different soil types.
   We thank the reviewer for his valuable suggestion. We agree that the study by Wang et al. (2017) gave valuable insight in the distribution of Se in various soils, and it was an oversight to not cite this study. Their findings have now been incorporated in the

Introduction and this whole paragraph has been substantially re-written to include more recent findings about the Se ageing process (lines 112-122).

6. Line 115-116, “This also allowed us to determine which chemical extraction method could predict Se bioavailability in soil most accurately.”, many studies have been done on this aspect, why should we repeat it? In addition, it isn’t suitable to use spiked soil to do such kind study. Since there are bigger difference between spiked soil and native soil. (Wang at al. Assessing the uptake of selenium from naturally enriched soils by maize *(Zea mays L.)* using diffusive gradients in thin-films technique (DGT) and traditional extractions. *Science of the Total Environment,* 2019,689:1–9)

   In areas of the world where soils have Se levels high enough to produce Se-rich foods, biofortification programs would not be necessary. Since the focus of the study was to ascertain the bioavailability of exogenous Se in soils with time, it was necessary to do so after naturally low-Se soils had been spiked. The reviewer is right in pointing out that previous studies have also carried out chemical extractions to predict Se availability, however, results differ according to the type of soils in which extractions are carried out. For example, Supriatin et al. (2016) showed that soil soluble organic Se, extracted by $CaCl_2$, correlated best with Se uptake in plants potentially because soils were rich in organic matter. In contrast, Li et al. (2016) showed that extracting soil Se with KH_2PO_4 gave a good prediction of the bioavailability of Se in soils. Dhillon and Dhillon (1999) observed that the extraction of acidic soils with KH_2PO_4 represented the bioavailable Se fraction more accurately than extraction with KCl, while the reverse was observed for basic soils. Given the lack of consistency in establishing a chemical method for the determination of Se bioavailability in soils, it was necessary to include this aspect in our study.

Material and methods

7. Table 1, if the sand content in soil is 98%, 95% or 85%, they are not soils. Thus the soils were selected without reprensentive.
   Two of the eight soils chosen in this study were sandy, with > 95% sand content, namely Mt Compass (pH 5.4) and Monarto (pH 8.9). According to a report by the government agency, Primary Industries and Regions SA (PIRSA, 2017), sandy soils (which roughly speaking are soils >90% sand; see texture triangle for details) form about 2.7 million hectares of the agricultural land in South Australia. Worldwide, sandy soils cover approximately 900 million ha (7% of the land area) and there are extensive areas of sandy soils under cultivation. We believe that the soils were carefully chosen, not only to showcase a range of physicochemical characteristics, but also to represent the different types of land available for agricultural use in Australia and worldwide. Hence, no changes were made to the text.

8. Line 147-148." labile or soluble Se (SeSol)extracted with 0.01 M calcium chloride (CaCl2)", why? Normal, phosphate extraction method are used.

   Although KH_2PO_4 has been previously used to extract the 'available Se' fraction in soils (Zhao et al., 2005), the use of $CaCl_2$ as a universal extractant, including for the determination of soil soluble Se fraction, is also an established reliable method (Houba et al., 2000; Supriatin et al., 2016). No changes made to the text.

9. Line 168, how many replicates?

   Four replicates were included for the pot trial and this information has now been added to the text (line 180).

10. Line 171-172, only one plant was left for each pot and grown up for six weeks, I was afraid that the biomass was not enough for analysis.

    All plants had biomass of > 0.5 g per pot, which was enough for the analysis of total Se in the plants following acid digestion (0.25 g per pot required). No changes made to the text.

11. Line 203, "Other elements (Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn) in plant samples were analysed"---why should we consider about it in this study?

    The elemental determination of plants, irrespective of treatments, is carried out as part of routine analysis. However, we agree that this information is not pertinent to the particular study and since these results have not been reported, we have removed this from the Materials and Methods section (line 216).

Results

12. "and was potentially also dissolving Se bound to carbonates."—how can you know it here?

    The possibility of a strong alkaline reagent such as TMAH dissolving carbonates-bound Se and contributing to the 'organically-bound Se' fraction of calcareous soils was a mechanism suggested to explain why the Se_{org} fraction of calcareous soils > Se_{org} of high-OM soils. For instance, it has been found that TMAH can react with dolomite (Chen et al., 2016). We do not claim an actual mechanism, but our giving a hypothesis for the observed results. Further research would be required to prove or disprove this hypothesis.

13. Table 4, "Non 1calcareous"—how do you classify it?

    Soils were classified as 'non-calcareous' if they had a pH < 7.0 and very low (below analytical detection limits) carbonate levels; on the other hand, alkaline soils (pH > 7.0) with detectable carbonate contents were classified as 'calcareous' soils. Kingaroy was classified separately, despite being an acidic soil, as it had high levels of iron oxides, behaved differently to the others and did not fit the model as explained in the text (Table 4).

14. Line 303-304, the $CaCO_3$ content is very low for the tested soils.
    Mallala was the soil with the highest $CaCO_3$ content (> 10%), while the other basic soils had a lower, albeit analytically detectable, carbonate content. Since carbonate content of soils seemed to be a determining factor in Se ageing, it was important to classify the soils in such a way as to make the distinction between calcareous and non-calcareous soils clear. No changes were made to the text.

15. Line 331-335, since only selenate was studied in this study, the comparation between selenate and selenite are not necessary here.
    The reviewer is right in pointing out that this study did not include a comparison between selenite and selenate. The text has been amended (lines 352-354).

16. Line 340-341," Compared to the number of studies about the sorption of Se ions on mineral oxides and clay surfaces"-- they are total different from soils.
    The text has been amended to discuss the sorption of Se on calcite surfaces only (line 358).

17. Line 342-343," studies investigating Se sorption in calcareous soils are fewer", please see the Wang 2017 as I mentioned above.
    The comparison between the number of studies on Se sorption on calcareous soils and non-calcareous soils has now been removed, because, as the reviewer pointed out earlier, this information was not pertinent to this study (line 358).

18. Line 417-420. "Our results were in agreement with those by Dhillon et al. (2005), in which the chemical extraction of Se from seleniferous soils (> 0.5 mg Se kg-1) by either hot water or potassium chloride (KCl), was more effective (r=0.70) in predicting bioavailability in wheat than its extraction by KH2PO4"--- It was suitable for high selenium content soil instead of the normal soil.
    The study by Dhillon et al. (2005) was quoted because it also showed the effectiveness of KCl extraction to predict bioavailability in soils containing high Se content; in Punjab, the soils are seleniferous (> 0.5 mg kg^{-1}), which is comparable to the soils in this study, which were spiked with 0.5 mg kg^{-1}. Even though most soils around the world contain lower levels of Se (0.05-0.1 mg kg^{-1}) (Lopes et al., 2017), certain parts of the world, for example in Punjab, soils contain higher levels of native Se (Dhillon and Dhillon, 1991). We believe that this statement highlighted the potential of using KCl or $CaCl_2$ as a chemical extraction method to predict bioavailability in high-Se and/or Se-amended soils in the field. No changes were made to the text.

19. Figure 2, what do read circle mean here?

    Due to a formatting error, a few of the data points were replicated on both graphs. Fig. 2 has been amended to show the differences in Se ageing between calcareous soils (top graph) and non-calcareous soils (bottom graph).

Reviewer #2

The MS presents the results of lab/greenhouse experiments performed to study the relationship between soil properties, contact period and availability of added selenate-Se by employing both chemical and biological methods. The topic of study has been properly introduced followed by a clear statement of objectives. The results and discussion sections have been described with suitable support from the published results and proper scientific reasoning. Language of the text is easily understood and free flowing. However, a few observations are recorded below:

- The abstract is slightly imbalanced in favour of experimental details as compared to the results. The basic objective was to understand the role of soil properties on Se ageing process. But the statement expressing the comparative role of different soil characteristics on the ageing process of Se is missing from the abstract.
  The implications of this study, which showed the effect of different soil properties on the ageing of added Se, have now been explicitly mentioned in the abstract (lines 29-30).

- As you know, the process of Se volatilization as DMSe is also an important aspect while studying Se transformation in soil. Measure of Se volatilization rates are necessary to provide a better understanding of Se fate in soil. This aspect is missing from the present studies. Comment.
  The reviewer is right in saying that the loss of Se from soils by volatilization is an aspect to consider to audit the fate of Se. However, since 99% of the applied Se had been recovered in the soils (from a total acid digest of the different soils) at the end of the incubation, minimal losses of Se by volatilization had occurred. Such information is available in the text at lines 257-258.

L 34-35 The statement "In recent years-------------" may be slightly modified. Because Se deficiency problem has been recognized quite earlier in some countries. Selenium containing fertilizers are being used for the last 4-5 decades to improve Se intake of the residents in Finland as well as New Zealand.
The text has now been amended accordingly (line 38).

L 38-39 The statement ending "-------human health", a suitable reference may be cited.
A suitable reference has now been added (line 43).

L 68-69 It must also be mentioned here that FA-Se complex is also liable to leaching beyond the root zone and may reach the water bodies
This information along with the suitable reference has now been added to the text (lines 73-77).

L 89-90 I feel this comparison is unnecessary. In fact, both the processes lead to Se immobilization
We believe that it was important to define the terms 'fixation' and 'ageing' in this paragraph to make the distinction between the two terms clear and avoid using

them interchangeably later in the text. While metal fixation refers to the immobilisation of metals in soils, as a result of chemical-physical and biological mechanisms, ageing refers to the time-dependent loss in metal solubility, which could be due to irreversible metal fixation. This paragraph has been amended to clarify the distinction between the two terms (lines 97-99).

L 95-96 The statement “On the other hand------------” is not true. For the first crop, Se^{VI} uptake by plants must be higher than Se^{IV}, but in the long term, residual value of Se^{IV} will be higher than Se^{VI}. No doubt Se^{VI} is more bioavailable, but it is also liable to leaching as well as subjected to reduction process in the long run.

We thank the reviewer for his insightful comments. The text has been amended to include more recent findings about the fate of Se^{VI} in aged soils, together with appropriate references (lines 104-111).

L 106-108 No doubt there are only a few published reports on this aspect of Se, but there exists another reference that may be included in this section if you find it suitable. The reference is --“Wang et al. (2019). Effects of straw amendment on selenium aging in soils: Mechanism and influential factors. Science of the Total Environment 657:871–881”

We thank the reviewer for his suggestion. This part of the text has been substantially re-written to include the findings from recent papers, including Wang et al. (2017) and Wang et al. (2019) (lines 112-122).

L 166 The title “Pot trial” looks like an old phrase and may be replaced with some more suitable title such as “Greenhouse experiment” etc.

The subtitle has now been changed to ‘Pot experiment’ (line 178).

L 178 The word “approximately” used here is not at all palatable. You must accurately and exactly weigh out the plant mass for Se analysis for sake of precision in analysis. Approximation means large variations in analysis and the data is not dependable.

The approximation was initially added because a range of 0.20-0.30 g (± 1% of 0.25 g) was acceptable for the analysis, provided the precise weight of the sample was recorded and used to calculate final plant Se concentrations. However, the reviewer is right in pointing out that the term ‘approximately’ is not really suitable given that the exact weight was recorded, so it has now been removed (line 191).

Table 1 For the last column, clarify the units of measurement

We thank the reviewer for spotting this error. The unit of measurement has been clarified (Page 9).

Fig 2. It is somewhat confusing. As per title, one part depicts calcareous soils and the second part non-calcareous soils. But actually data for both groups of soils are depicted in both parts of the figure. Needs modification or clarification in the title.

Due to a formatting error, a few of the soils appeared on both graphs. Fig. 2 has now been modified to clearly depict the changes in Se solubility with time for calcareous (top graph) and non-calcareous soils (bottom graph).

References

Buekers, J., Degryse, F., Maes, A., Smolders, E., 2008. Modelling the effects of ageing on Cd, Zn, Ni and Cu solubility in soils using an assemblage model. Eur. J. Soil Sci. 59(6), 1160-1170.

Chen, B., Deng, M., Lan, X., 2016. Behaviours of reactive silica and dolomite in tetramethyl ammonium hydroxide solutions., Proc. of the 15th Int. Conf. on Alkali-Aggregate Reactions in Concrete. The Concrete Society, St Paul, Brazil, pp. 245-254.

Dhillon, K.S., Dhillon, S.K., 1999. Adsorption–desorption reactions of selenium in some soils of India. Geoderma 93(1), 19-31.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., Vark, W.v., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun Soil Sci Plant Anal. (9-10), 1299-1396.

Li, J., Peng, Q., Liang, D., Liang, S., Chen, J.S., Sun, H., Li, S., Lei, P., 2016. Effects of aging on the fraction distribution and bioavailability of selenium in three different soils. Chemosphere 144, 2351-2359.

PIRSA, 2017. Sandy soils. Primary Industries and Regions SA (PIRSA), South Australia.

Supriatin, S., Weng, L., Comans, R., 2016. Selenium-rich dissolved organic matter determines selenium uptake in wheat grown on low-selenium arable land soils. Plant and Soil 408(1), 73-94.

Tye, A.M., Young, S., Crout, N.M.J., Zhang, H., Preston, S., Zhao, F.J., McGrath, S.P., 2004. Speciation and solubility of Cu, Ni and Pb in contaminated soils. Eur. J. Soil Sci. 55(3), 579-590.

Wang, D., Zhou, F., Yang, W., Peng, Q., Man, N., Liang, D., 2017. Selenate redistribution during aging in different Chinese soils and the dominant influential factors. Chemosphere 182, 284-292.

Wang, M., Cui, Z., Xue, M., Peng, Q., Zhou, F., Wang, D., Dinh, Q.T., Liang, D., 2019. Assessing the uptake of selenium from naturally enriched soils by maize (*Zea mays* L.) using diffusive gradients in thin-films technique (DGT) and traditional extractions. Sci. Total Environ. 689, 1-9.

Zhao, C., Ren, J., Xue, C., Lin, E., 2005. Study on the relationship between soil selenium and plant selenium uptake. Plant and Soil 277(1), 197-206.

Effect of soil properties on time-dependent fixation (ageing) of selenate

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1 Abstract

Previous biofortification studies have established that the residual effect of added selenium (Se) fertilisers on second-season crops is minimal. To explore the fate of exogenous Se in soil, chemical and biological methods were employed to assess the change in Se bioavailability with time. Eight soils varying in physicochemical properties were spiked with sodium selenate (0.5 mg kg^{-1} Se) and incubated at 25°C for different periods (1, 30, 60, 90 and 300 d). At the end of the incubation, soil Se was fractionated by a sequential extraction procedure into 'soluble', 'adsorbed' and 'organically-bound' Se fractions. Simultaneously, wheat was grown in the Se-aged soils, under controlled conditions for six weeks, and uptake was determined. A general decrease in Se solubility over time was observed, but the rate of decrease varied depending on soil type. A reversible first order model fitted the Se ageing kinetics well, except in an Oxisol. The most pronounced ageing was observed in calcareous soils. Concentrations of Se in the shoots of wheat grown in freshly spiked soils ranged from 71.8 ± 17.5 mg kg^{-1} in calcareous soils to 110 ± 31.6 mg kg^{-1} in non-calcareous, low-OM soils. With ageing, shoot Se concentrations decreased to < 10 mg kg^{-1}, with the lowest concentrations (0.31 ± 0.03 mg kg^{-1}) measured in plants grown in calcareous soils. The Se concentrations in plants correlated well with the soluble Se fraction (extracted with 0.01 M $CaCl_2$). The findings of this study can be used to make more informed decisions about the rate and frequency of Se fertiliser application in agronomic biofortification programs.

Keywords: selenate; ageing; chemical extractions; pot trial; modelling

Highlights

- Ageing of Se was more pronounced in calcareous soils than non-calcareous ones.
- Selenium ageing was well represented by a reversible first order equation.
- Chemical extraction of soils with $CaCl_2$ was effective in predicting Se bioavailability.

2 Introduction

Selenium (Se) is an essential nutrient required for proper functioning of the human immune system. In the last four decades, a decline in human dietary Se levels has triggered research into ways by which Se consumption can be increased in a sustainable manner (Haug et al., 2007). Applying Se fertilisers to staple crops, a process known as biofortification, is highly effective in increasing Se concentrations in edible crop parts, such as wheat grain, destined for human nutrition (Gupta and Gupta, 2000). However, excessive dietary intake of Se can also be detrimental to human health (Rayman, 2012). It is therefore vital to understand how applied Se is transferred within the soil-plant system and determine how factors such as soil conditions and contact time influence that transfer.

Inorganic Se exists mainly as anionic oxy-acid species in most agricultural soils, in the form of selenate (SeO_4^{2-} or Se^{VI}) and selenite (SeO_3^{2-} or Se^{IV}) (Banuelos and Schrale, 1989; Gupta and Gupta, 2000). In alkaline aerobic soils, Se^{VI} tends to predominate while in more reducing acidic conditions, Se^{IV} is usually the prevalent inorganic species (Elrashidi et al., 1987). Selenium ions have different sorptive behaviour in soil, which also results in a difference in their mobility. Anions are adsorbed onto soil geocolloidal phases by several mechanisms, including inner- and outer-sphere complexation. Outer-sphere complexes, formed by weak electrostatic forces of attraction between ions and the functional groups of soil sorbent phases, are usually reversible (Bowden et al., 1980). On the other hand, inner-sphere complexation, which occurs by covalent bonding between the ions and the functional groups, results in stronger sorption behaviour. Selenite ions are adsorbed specifically by inner-sphere complexation onto surfaces of soil components such as oxides of aluminium (Al) and iron (Fe) as well as clay mineral edges (Peak, 2006; Zhang and Sparks, 1990). Selenate, on the other hand, tends to form mostly outer-sphere complexes, which explains its lower sorption and higher mobility compared to Se^{IV} in soils (Peak and Sparks, 2002; Sparks, 2003). Soil pH is a major factor influencing Se mobility through its influence on the surface charge of minerals and the speciation of Se oxyanions. For example, greater sorption of Se^{IV} onto soil mineral phases is observed under acidic, compared to neutral/alkaline, conditions (Dhillon and Dhillon, 1999; Neal et al., 1987). Studies by Gissel-Nielsen and Hamdy (1977) also highlighted the importance of the mineral phases present on the Se sorption capacity of soils; for example, the adsorption of selenite on 2:1 aluminosilicate minerals such as montmorillonite is greater than on 1:1 minerals such as kaolinite, due to the greater surface area available for anion sorption (Frost and Griffin, 1977). The presence of soil organic matter (SOM) also influences Se bioavailability (Li et al., 2017). Depending on the type of complexes formed between Se and SOM fractions, Se is irreversibly immobilised or weakly retained (Smažíková et al., 2017). As described by Qin et al. (2012), Se is more weakly bound to fulvic acids (FA) compared to humic acids (HA). Given the greater solubility of FA, there is also the potential for FA-bound Se to dissolve in soil pore water and become bioavailable or even leach out the of root zone to nearby water bodies, potentially becoming a source of contamination to aquatic biota. Leaching of weakly-bound organic Se was posited as an explanation for a sudden surge in human selenosis cases in a high-Se area in China (Qin et al., 2012).

The total concentration of Se in soil is not a good indicator of its bioavailability (Gupta and Gupta, 2000). Instead, chemical extraction methods (single or sequential) can be employed to fractionate Se in soils and may provide a better estimate of Se bioavailability. ‘Soluble’ Se is usually extracted using hot water or a dilute salt solution based on anion exchange (Wright et al., 2003), while ‘adsorbed’ Se can be extracted using a phosphate solution through ligand exchange; phosphate adsorbs more strongly than selenite on soil surfaces (Hingston et al., 1967; Keskinen et al., 2009). The organically-bound Se fraction is rather difficult to separate from inorganic Se species, but alkaline reagents such as sodium hydroxide (NaOH) and tetramethylammonium hydroxide (TMAH) solubilise organic matter (OM) and release OM-bound Se (Hingston et al., 1967). An advantage of TMAH over inorganic extractants such as NaOH is that pH can be increased without concurrently increasing the salt concentration of the solution, which can limit the sensitivity of inductively coupled plasma mass spectrometers (ICP-MS) typically used for Se analysis (Hassan, 2011). Although laborious and operationally defined, sequential extraction procedures (SEPs) are useful to fractionate analytes and can be used to assess changes in extractability over time.

When metals are added to the soil, their solubility decreases towards an asymptotic equilibrium – as a result of fixation reactions in the soil (Buekers, 2007). The fixation mechanisms of trace metals such as Pb, Zn, Cu and Co have been explored thoroughly (Barrow, 1998; Degryse et al., 2009) while those of Se are still relatively poorly understood. 'Fixation' hereby refers to the immobilisation process of Se that may occur progressively for days and years following initial rapid (hours) adsorption of Se in soil (Buekers, 2007); ageing refers to the time-dependent process of Se fixation in the soil. Studies about the fixation behaviour of Se showed that the chemical speciation of the anions influences their retention capacity in the soil (Barrow and Whelan, 1989): Se^{IV} is initially adsorbed and is then further retained, possibly by diffusive penetration into soil particles such as Fe oxides. This suggests that Se^{IV} continues to react with the soil for a very long time, with little desorption, and hence become increasingly unavailable to plants. Selenate, on the other hand, has been found to remain more bioavailable in soils in the short term, especially in neutral to alkaline soils, as it is weakly adsorbed onto soil particles. However with time, Se^{VI} may be reduced to lower valence state species such as Se^{IV}, Se^0 and Se^{2-}, leading to further adsorption of the reduced species onto surfaces such as carbonate and Fe/Mn oxides or incorporation into humus. This effect is slower in neutral to alkaline soils, compared to acidic soils with high Fe/Mn oxides and OM content, where faster reduction of Se^{VI} to Se^{IV} occurred (Wang et al., 2017).

Using sequential chemical extractions to partition Se in Se^{VI}-spiked soils over a period of a year, Wang et al. (2017) showed that the rate of Se ageing is primarily dictated by soil pH, Fe and Al oxides and SOM. More recently, Wang et al. (2019) and Peng et al. (2019) used the diffusive gradient thin (DGT) film technique to assess the change in bioavailable Se with time. The DGT technique can mimic the dynamic movement of Se in a soil-plant system, including the re-supply of the solution phase with adsorbed Se, but can be quite costly and labour-intensive. To validate the effectiveness of chemical extraction methods in predicting bioavailability in a cost-effective manner, biological assays need to be undertaken simultaneously. Li et al. (2016) used a combined approach of sequential extractions and a crop trial to assess the ageing of Se in soil. However, their study only comprised three soils and so could not relate Se ageing to soil characteristics.

Our study addressed this gap by combining chemical, biological and predictive modelling techniques to gain a holistic understanding of the fate of added selenate fertiliser in eight soils with contrasting soil properties over a period of time (300 d) which exceeded a typical growing season. The main soil properties driving the ageing of the added Se were determined. Such information will be crucial in assessing the residual value of Se^{VI} fertilisers in different soil systems, which could be used to make more informed decisions about Se fertiliser application rates in biofortification programs.

3 Materials and Methods

3.1 Soils

Eight Australian soils were chosen to represent a range of physicochemical properties that were likely to affect Se dynamics. The soils were air-dried, sieved to < 2 mm and homogenised prior to the experimental setup. The physical and chemical properties of the soils are listed in Table 1. Briefly, soil pH and electrical conductivity (EC) were determined in a 1:5 soil-to-water suspension; the effective cation exchange capacity (ECEC) was determined by the ammonium acetate method in a 1:10 soil-to-solution ratio at pH 7 (Rayment and Higginson, 1992); the $CaCO_3$ content was determined by a manometric procedure (Martin and Reeve, 1955); total organic carbon (C) (TOC) was quantified by a dry combustion method (Matejovic, 1997); the oxalate Al and Fe contents were determined according to Rayment and Higginson (1992); the textural classification of the soils was determined using a mid-infrared spectroscopy method (Janik et al., 2016); available phosphorus (P) and sulphur (S) in the soil were measured after extraction with sodium bicarbonate and potassium chloride solutions respectively (Rayment and Lyons, 2011).

Table 1: The physicochemical characteristics of the soils used in this study. 'b.d' was used to denote concentrations that were below analytical detection limits.

Soils	**pH**	**EC**	**ECEC**	**$CaCO_3$**	**Org C**	**Oxal Al**	**Oxal Fe**	**Clay**	**Sand**	**Available P**	**Available S**	**Total Se**
	water	**dS m^{-1}**	**c.mol kg^{-1}**	**%**	**%**	**g kg^{-1}**	**g kg^{-1}**	**%**	**%**	**mg kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**
Mt Compass	5.4	0.01	1.7	b.d	0.2	0.1	0.1	1.0	98	5	3	0.02
Inman Valley	5.6	0.14	16.0	b.d	5.7	1.4	5.2	26.0	41	41	19	0.18
Charleston	5.8	0.09	7.8	b.d	5.1	0.4	4.6	3.6	85	190	14	0.23
Kingaroy	6.8	0.09	8.3	b.d	1.9	2.5	3.7	52.0	20	10	61	0.13
Balaklava	8.0	0.17	27.0	3.1	1.7	1.2	0.8	12.0	46	35	13	0.10
Black Point	8.0	0.12	15.9	2.7	1.1	1.0	0.8	15.0	70	5	10	0.10
Mallala	8.2	0.13	25.9	11.0	0.9	1.2	0.9	13.0	62	16	9	0.14
Monarto	8.9	0.09	5.1	1.1	0.4	0.3	0.2	2.6	95	5	5	0.05

3.2 Soil spiking and incubation

All soils were spiked with 0.5 mg Se kg^{-1} in the form of sodium selenate (Na_2SeO_4). After spiking, the soils were watered to 60% water holding capacity (WHC) and mixed to ensure homogeneity. They were left to equilibrate overnight and then split into four replicates of 500 g in plastic containers. The soils were incubated under aerobic conditions at 25°C. . During the incubation period, soils were watered regularly to maintain 60% WHC. Control soils (no added Se) were also included. The soils were incubated for 1, 30, 60, 90 and 300 d prior to extraction and plant growth assays.

3.3 Soil extractions

After incubation, the soils were dried in an oven at 40°C for 72 hours then ground to a homogenous fine powder prior to analytical procedures. Sequential extraction was carried out to determine the partitioning across different fractions, namely: soluble Se (Se_{Sol}) extracted with 0.01 M calcium chloride ($CaCl_2$), adsorbed Se (Se_{Ads}) extracted with 0.016 M potassium dihydrogen phosphate (KH_2PO_4) and organically-bound Se (Se_{Org}) extracted using 10% TMAH, adapted from methods developed by Wright et al. (2003), Supriatin et al. (2016) and Mathers et al. (2017). Briefly, 0.5 g of soil was shaken on an end-over-end shaker with 5 mL of extractant at 100 rpm overnight, followed by centrifugation at 3000 *g* for 30 minutes and finally syringe-filtration of the supernatant through 0.45 µm filters into 10-mL tubes. In between the extractions, the weight of the soil samples was recorded to account for the amount of Se carried over from the previous extraction. A separate single Se extraction by *aqua regia* digestion, adapted from the British standard method ISO 11 466, was also carried out to quantify pseudo-total Se in the soil (Se_{Tot}): 0.5 g of soil sample was cold digested overnight with concentrated hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a ratio of 3:1:1. Subsequently, the samples were digested at 125°C for 4 hours in a closed system on a block digester. All samples were made up to 50 mL volume with Milli-Q water and stored at 5°C in polypropylene bottles pending analysis.

The method used for the single *aqua regia* extraction was verified through the use of the certified reference material NCS DC 73326 from the China National Analysis Centre for Iron and Steel (Beijing China, 2008). Results were within 10% of the certified value (1.6 ± 0.2 mg Se kg^{-1}), with a mean of 1.64 ± 0.15 mg Se kg^{-1}.

3.4 Pot experiment

In parallel to chemical extractions, wheat (*Triticum aestivum cv.* Axe) was grown in 250 g pots filled with the incubated soils, with four replicates. Prior to potting, the soils were mixed with a basal nutrient solution supplying the following nutrients (mg kg^{-1} of soil): N (80, split application), P (20), K (40), S (40), Ca (10), Mg (10), B (1.0), Cu (2.0), Mn (2.0), Mo (0.1) and Zn (2.0), and left to equilibrate overnight. After potting, two pre-germinated wheat seedlings were sown into the pots and thinned to one plant per pot two weeks later. The soils were watered regularly to maintain soil moisture at 60% WHC. Wheat was grown under controlled conditions (temperature of 23.2°C, humidity of 72% and 12 h daylight cycle) for six weeks.

The aboveground biomass of the wheat plants was hand-harvested and dried in an oven at 50°C for 72 h, or until a constant weight was achieved. The dried samples were finely ground using a laboratory-grade grinder and stored dry prior to total Se analysis. The plant Se concentration (Se_{Plant}) was determined after acid digestion:0.25 g of plant sample (four replicates) was weighed into 50-mL digestion tubes (Axygen, Thermo Fisher Scientific, New York) and left overnight in 2 mL of HNO_3 acid and 0.5 mL of H_2O_2 to predigest. The samples were then heated to 80°C for 45 min, followed by 125°C for 160 min on a block digester. After acid digestion, the samples were cooled for 30 min then made to 20 mL volume using ultrapure Milli-Q water (18.2 MΩ cm).

3.5 Sample analysis

Concentrations of total Se in soil and plant samples were determined using an inductively coupled plasma – optical emission spectrometer, ICP-OES (Optima 4300, Perkin Elmer) fitted with a continuous flow hydride generator (Hyd-ICPOES). Since only Se^{IV} is reduced to a hydride, a pre-reduction step of Se^{VI} to Se^{IV} was carried out: 3 mL of the different Se extracts ($CaCl_2$, KH_2PO_4 and TMAH) were heated with an equal volume of HCl at 90°C for 30 minutes, prior to analysis. All standards and blanks were subjected to the same treatment before injection into the Hyd-ICPOES instrument.

For data quality control, detection limits were calculated for each extraction method as three times the standard deviation of the blank measurements (Shrivastava and Gupta, 2011). Concentrations of Se above the highest calibration standard were re-analysed after a 10- or 100-fold dilution of the sample with Milli-Q water. The detection limits for Se were: 0.19, 0.15, and 0.18 µg L^{-1} for $CaCl_2$, KH_2PO_4, TMAH extractions, respectively. Due to the consistently low recovery of the Se_{Org} fraction by this method, a separate single TMAH extraction, using the same protocol as above, was repeated on all soil samples and the extracts were analysed for total Se concentrations by ICP-MS (Thermo-Fisher iCAP-Q) with H_2 gas added to the collision cell at a flow rate of 4 mL min^{-1}. The Se_{Org} fraction in the present study was therefore calculated as the difference between total Se extracted by the single separate TMAH extraction (Se_{TMAH}) and the sum of soluble and adsorbed Se (Se_{Sol} + Se_{Ads}).

3.6 Kinetics models

Several kinetic models were tested for their ability to represent the change in Se bioavailability, here represented by the Se_{sol} fraction in selenate-spiked soils, with time (Table 2).

Table 2: Kinetics models for Se ageing in different soils (Boostani et al., 2019; Islas-Espinoza et al., 2014; Li et al., 2016).

Models	Equations	Parameters
Reversible First Order	Eq.1	See text
Second order	$\frac{1}{Se_t} = \frac{1}{Se_{eq}} + k_2 t$	Se_t is the concentration of Se_{Sol} (mg kg^{-1}) at specific ageing time, t (days); Se_{eq} is the concentration of Se_{Sol} at equilibrium (mg kg^{-1}); k_2 is the second-order rate constant
Power function	$\ln Se_t = \ln a + \mathrm{b} \ln t$	a and b are both constants
Elovich equation	$Se_t = a + b \ln t$	
Parabolic diffusion	$\frac{Se_t}{Se_{eq}} = a + b\sqrt{t}$	

The most comprehensive model, the Reversible First Order (RFO) – Eq. 1 – was adapted from Buekers et al. (2008) and Crout et al. (2006) as follows:

$$f = f_{eq} + \left(f_0 - f_{eq}\right) \times e^{-t/T_c} \qquad \text{Eq. 1}$$

where f, f_0 and f_{eq} are the fractions of added Se that are soluble at time t, time 0 and at equilibrium, respectively, and T_c is the response time of the reaction (d).

All model parameters were optimised by minimising the residual standard deviations (RSD) (Eq. 2). The goodness-of-fit of the models to the selenate ageing process was assessed using a minimal RSD and the Pearson's correlation coefficients (r) generated from correlating the measured and modelled data. The RSD was calculated as follows:

$$RSD = \sqrt{\frac{\Sigma(Se_{meas} - Se_{model})^2}{n - x}} \qquad \text{Eq. 2}$$

where Se_{meas} and Se_{model} were the measured and modelled values of Se_{Sol} (mg kg^{-1}), respectively; n is the number of data points, and x is the number of model parameters.

3.7 Statistical analyses

The changes in Se concentrations in various soil fractions over time were assessed for statistical significance using a repeated measures Analysis of Variance (ANOVA) with Greenhouse-Greisser correction. A Bonferroni Post Hoc test was carried out to determine the time points at which Se solubility in the soils changed significantly.

Eleven soil properties (Table 1) were used to assess the main drivers of Se ageing, represented by the fraction of Se that was soluble in the soil at equilibrium (f_{eq}), in a stepwise linear regression. The data was assessed for multicollinearity using a variance inflation factor (VIF) check. No correlation was observed between the properties (VIF < 2.0) (Dodge, 2008).

Using a simple correlation analysis, the effectiveness of chemical extraction methods to predict Se bioavailability (plant Se concentrations) was assessed. The statistical analyses were conducted in SPSS (IBM SPSS Statistics for Windows, Version 24.0., IBM Corp, Armonk, New York). The parameters of the different kinetics models were fitted to the data using the Microsoft Excel Solver function.

4 Results and Discussion

4.1 Change in soil Se fractions with ageing

The background concentrations of total Se in the soils used in this study ranged from 0.02 – 0.23 mg kg^{-1}, with the highest concentrations observed in soils with high organic matter content, such as Charleston and Inman Valley (9-10% OM, assuming a conversion factor of 1.72 from organic C) (Howard, 1965) (Table 1). Total Se concentration of the aged soils averaged 0.50 ± 0.01 mg kg^{-1}, which was 99 ± 1.6% recovery of the added Se (Se$_{\mathrm{Add}}$) after correction for background Se in soil. This suggests that limited losses of Se by volatilisation occurred during the incubation period.

The solubility of Se generally decreased over time, but at various rates depending on the type of soil (Fig. 1). Sandy soils such as Monarto and Mt Compass showed no statistically significant change ($P > 0.05$) in Se$_{\mathrm{Sol}}$ over 300 d, as the % Se$_{\mathrm{Add}}$ that was soluble decreased from 96% to 71% in Monarto and from 103% to 68% in Mt Compass. For the other soils, a rapid initial decrease in Se$_{\mathrm{Sol}}$ was observed, followed by a more gradual partitioning into non-labile pools. The most rapid decrease in Se$_{\mathrm{Sol}}$ occurred in Kingaroy, where the majority of the Se$_{\mathrm{Add}}$ (> 50%) was not extractable by $CaCl_2$ within the first 24 h. The Kingaroy soil is rich in clay (52%) and Al and Fe oxides (2.54 and 3.69 g kg^{-1} respectively), explaining its high Se retention capacity. The second fastest decrease in Se$_{\mathrm{Sol}}$ was observed in the alkaline calcareous Mallala soil (11% $CaCO_3$), as solubility decreased by 95% within the first 30 d, followed by a more gradual decrease. These results compare well with those in studies by Singh et al. (1981), who showed greater Se retention in calcareous soils compared to non-calcareous, saline and alkaline soils.

The Se_{Ads} fraction showed a similar pattern of distribution in most soils (Fig. 1). A slight increase in Se_{Ads} was observed for the first 60 d, followed by a decrease and stabilisation over a longer-term. Except for the Kingaroy soil, Se_{Ads} was small at d 1 (7 ± 0.88% of Se_{Add}), increased to 16 ± 2.35% at d 30 and gradually decreased to 6 ± 0.95% at d 300, showing that Se_{Ads} was not the dominant fraction in those soils. This trend suggests that Se was immobilised by processes which retained the Se more strongly than surface adsorption. Comparatively, Kingaroy was the only soil where Se_{Ads} was higher than Se_{Sol} at every ageing time, which suggests that specific adsorption onto mineral soil fractions was the primary mechanism responsible for the loss of solubility in the Oxisol (Fig. 1). A similarly high ratio of Se_{Ads}:Se_{Sol} was observed in soils with comparable mineral oxides contents to Kingaroy in a study by Li et al. (2016). This highlights the efficacy of active sites such as those of Al and Fe oxides as well as clay minerals to rapidly adsorb Se, whether supplied as soluble Se^{VI} or Se^{IV}.

At d 1, Se_{Org} averaged 0.17 ± 0.05 mg kg^{-1} in most soils, which gradually increased with ageing time (Fig. 1). High-OM soils such as Inman Valley and Charleston were expected to have higher Se_{Org} fractions compared to others; however, Se_{Org} was the greatest in the alkaline calcareous soils Mallala, Black Point and Balaklava, suggesting that TMAH did not specifically solubilise organically-bound Se but also dissolved Se bound to carbonates. The determination of organically-associated Se often requires the use of highly alkaline reagents, such as NaOH, or oxidising agents such as sodium hypochlorite (NaOCl) and potassium persulfate ($K_2S_2O_8$). However, these reagents can lead to an overestimate of the organically-bound fraction if they release Se bound to other fractions in the soil. For example, Wright et al. (2003) found that the NaOCl extraction of sediments resulted in overestimation of the Se_{Org} fraction, as the extract was also oxidising/solubilising metal selenides such as iron selenide (FeSe). Moreover, if the phosphate extraction (for Se_{Ads}) did not strip off all surface-bound Se^{IV}, the TMAH extraction would probably desorb the remaining Se^{IV} due to surface charge and ligand exchange mechanisms. Again, this could lead to an overestimation of the Se_{Org} fraction.

4.2 Kinetics models for Se ageing in soils

For all soils, except Kingaroy, the best fit of the data to the selenate ageing process was given by the reversible first order (RFO) equation model. Kingaroy was best modelled by a Power Function (Table 3). Given that the RFO model gave the best fit for the majority of the soils, it will be the only one discussed further.

Table 3: Residual standard deviation (RSD) and Pearson's correlation coefficients (r) of kinetics models for change in soluble Se fraction over time in different soils. The numbers highlighted in bold show the best goodness-of-fit between modelled and measured Se_{Sol} values.

Kinetic models	Mt Compass		Inman Valley		Charleston		Kingaroy		Balaklava		Black Point		Mallala		Monarto	
	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r
Reversible first order	**0.04**	**0.88**	**0.01**	**0.99**	**0.08**	**0.91**	0.11	0.00	**0.05**	**0.99**	**0.04**	**0.94**	**0.00**	**1.00**	**0.01**	**0.98**
Second order	0.04	0.80	0.06	0.73	0.10	0.75	0.09	0.00	0.06	0.95	0.14	0.73	0.01	1.00	0.01	0.97
Power Function	0.06	0.00	0.02	0.98	0.22	0.79	**0.08**	**0.55**	0.10	0.88	0.12	0.77	0.01	1.00	0.04	0.45
Elovich	0.06	0.00	0.01	0.98	0.08	0.79	0.09	0.00	0.07	0.94	0.08	0.94	0.08	0.93	0.04	0.57
Parabolic Diffusion	0.06	0.67	0.06	0.75	0.14	0.62	0.09	0.00	0.13	0.86	0.17	0.86	0.20	0.57	0.02	0.95

Table 4: The estimated model parameters derived from the reversible first order model to predict Se solubility (Se_{Sol}) over time. Kingaroy was not well represented by the model.

Soils	Soil type	f_0	f_{eq}	T_c (d^{-1})
Mallala	Calcareous	0.97	0.01	9.40
Balaklava		1.00	0.02	48.3
Black Point		0.98	0.01	110
Monarto		0.89	0.00	943
Inman Valley	Non calcareous	0.80	0.44	18.9
Charleston		0.84	0.23	56.5
Mt Compass		0.98	0.66	121
Kingaroy	Oxisol	0.25	-	-

The RFO model parameters indicate whether there was immediate sorption of Se onto soil surfaces ($f_0 < 1$), how much of the added Se remains soluble at equilibrium (f_{eq}) and the 'response time' of the ageing reaction (T_c, the time required to reach 63% of the difference between the initial and equilibrium values) (Table 4). The capacity of soils to instantly 'fix' Se ($1-f_0$) was not well correlated with Se ageing. For example, alkaline calcareous soils such as Mallala, Balaklava and Black Point, which had a low proportion of immediately-fixed Se (< 5%), showed more pronounced Se ageing (higher f_{eq}) compared to other soils (Table 4). For the calcareous soils, almost all of the added Se was predicted to be fixed at equilibrium, and this fraction increased with increasing $CaCO_3$ content of soils, following the order of Mallala ($CaCO_3$ = 11%) > Balaklava ($CaCO_3$ = 3.1%) > Black Point ($CaCO_3$ = 2.7%) > Monarto ($CaCO_3$ = 1.1%) (Fig. 2).

Low instantaneous immobilisation of added Se in the calcareous soils suggest that more Se remained bioavailable shortly after addition probably because the alkaline, aerobic soil conditions favour the predominance of mobile Se^{VI} ions (Gupta and Gupta, 2000).

However, over time, more pronounced Se ageing occurred in the calcareous soils (Fig. 2), pointing to a strong binding mechanism, possibly by ion exchange with CO_3^{2-} on calcite surfaces (Goldberg and Glaubig, 1988). The non-calcareous soils showed relatively less pronounced ageing of Se (Table 4), and the rate of Se fixation in high-OM soils such as Inman Valley and Charleston (T_c of 19 and 57 d) was greater than that in low-OM soils such as Mt Compass and Monarto (T_c of 120 and 942 d respectively). Despite the low $CaCO_3$ content (1.1%) of Monarto, its f_{eq} was predicted to be 0 (Table 4), which suggests that Se fixation in Monarto would occur at a very slow rate over a long period of time (estimated 942 d). Given that the ageing period for this study was 300 d, it was not possible to verify this prediction. However, for all the other soils, f_{eq} was estimated to be reached within the 300 d period ($T_c < 300$; Table 4) and the good correlation observed between f_{eq} and Se_{Sol} concentrations at d 300 for those soils (r = 0.99) (data not shown) suggests that the model prediction was reliable.

While the RFO model described the pattern of ageing in most soils well, it did not indicate which mechanisms drove Se ageing. Moreover, given the lack of selectivity of certain extractants, such as TMAH, it was not possible to verify the processes that may have caused these changes. It was only possible to speculate on the basis of apparent correlations with soil properties. For example, the retention of Se onto calcite surfaces by an ion exchange mechanism may explain the more pronounced retention of Se in calcareous soils.

The Oxisol, Kingaroy, was not well represented by kinetic models because most sorption occurred in the first day (> 75% of the added Se adsorbed with 24 h) and there was little change in Se solubility afterwards. This soil was, therefore, omitted from the statistical analysis determining which soil fractions were primarily driving Se ageing.

4.3 Effect of soil properties on Se ageing

A multiple linear regression analysis between the estimated Se_{Sol} fraction at equilibrium (f_{eq}) and soil properties showed that soil pH was the main property influencing the loss in Se solubility with time, explaining 81% of the variance among the different soils ($p < 0.05$) (Fig. 3). Under aerated alkaline conditions, Se^{VI}, which is weakly retained on soil particles, is expected to predominate (Mayland et al., 1991). Hence, the more pronounced ageing at higher pH may seem surprising. However, the alkaline soils were also calcareous, and the rate of Se fixation increased with increasing $CaCO_3$ content of the soils. It seems therefore likely that retention of Se on $CaCO_3$ was the primary mechanism responsible for Se ageing in the alkaline soils.

The sorption of Se^{VI} in calcareous soils has not been studied extensively (Cowan et al., 1990; Jones and Belling, 1967; Renard et al., 2013; Singh et al., 1981). Goldberg and Glaubig (1988) showed that the retention behaviour of Se on calcite, especially Se^{IV}, was highly dependent on soil pH as sorption increased from pH 6 to 9, peaked between pH 8 and 9, and decreased at pH values > 9. Studies have used the chemical similarity of sulphate (SO_4^{2-}) and phosphate (HPO_4^{2-}, PO_4^{3-}) ions with Se^{VI} and Se^{IV}, respectively, to infer information about, and better understand, the mechanisms governing Se retention in calcareous soils (Barrow and Whelan, 1989). For example, Cowan et al. (1990) showed that, in a similar manner to HPO_4^{2-}, Se^{IV} was adsorbed on $CaCO_3$ by its exchange with CO_3^{2-}/HCO_3^- ions on anion exchange surface sites of calcite. This could be explained by the fact that both Se^{IV} (SeO_3^{2-}) and HPO_4^{2-} have similar charge, ionic size and structure (Shock and Helgeson, 1988). However, it should be noted that PO_4^{3-} was applied to the soil at a much higher concentration than Se^{IV}, which is representative of field conditions. In the field, P application is usually > 20 kg P ha^{-1}, which is > 2000 times the Se application rate (Heming, 2007). It can be argued that the precipitation of Se^{IV} ions at such low levels on calcite surfaces would be unlikely. In the case of Se^{VI}, structural studies using X-ray Absorption Fine Structure (XAFS) spectroscopy showed direct evidence of SeO_4^{2-} ions substituting for CO_3^{2-} in calcite; however, high concentrations of aqueous Se (1006 mg L^{-1}) was used in that study as well (Lamble et al., 1995). It was suggested that, although retention of Se onto calcite surfaces *via* an ion exchange mechanism seems possible, it would require substantial distortion of the CO_3^{2-} site to accommodate the large SeO_4^{2-} ions (Reeder et al., 1994). More studies are hence required to investigate the potential retention mechanism of Se onto calcite when Se is added at a realistic application rate to the soil.

In non-calcareous soils, initial (inorganic) Se retention occurs predominantly by an adsorption mechanism (Goldberg, 2014) and adsorption strength is, in turn, primarily dictated by pH and the contents of Fe/Mn and Al oxides as a result of their specific surface area and strong chelating ability (Dinh et al., 2019; Muller et al., 2012). In this study, the most rapid decline of Se_{Sol} (> 75% within 24 h) was observed in the Kingaroy soil (Fig. 1), which is an Oxisol with large Al/Fe oxide and clay contents (Table 1), suggesting that the oxides were responsible for Se sorption in this soil. In the other non-calcareous soils, ageing was most pronounced in the high-OM soils (Fig. 2), suggesting that binding onto, or incorporation into, OM played a role in the ageing process. However, it is hard to infer the type of bonding responsible for the retention of Se in OM. The chemical fractionation method employed in this study does not even differentiate between HA- and FA-bound Se; furthermore, extraction under alkaline conditions may include intact organic-Se species and inorganic Se released by alkaline hydrolysis.

4.4 Predicting availability of Se to plants

The Se concentrations of plants grown in soils aged with Se for different lengths of time over a 300 d period are shown in Table 5. The plant Se concentrations generally declined with time of incubation after Se addition, but the rate of decrease varied significantly among the different soils. Initial (t=30 d) plant Se concentrations were highest in non-calcareous, low-OM (sandy) soils (average 110 ± 31.6 mg kg^{-1}), followed by calcareous soils (average 71.9 ± 17.5 mg kg^{-1}) and were lowest in high-OM soils (average 17.3 ± 6.74 mg kg^{-1}). By the end of the ageing period, plant Se concentration decreased to < 10 mg kg^{-1} in all soils, with the exception of sandy soils (Se_{Plant} average of 58.3 ± 1.40 mg kg^{-1}). The lowest concentrations at the end of the experiment were measured in plants grown in the calcareous soils Mallala, Black Point and Balaklava (average of 0.45 ± 0.03 mg kg^{-1}) (Table 5).

To determine which chemical extraction method was the best indicator of bioavailability, concentrations of Se in plants were correlated with Se_{Sol} and 'plant-available' Se (Se_{Sol} + Se_{Ads}) fractions (Fig. 4). A dilute salt (0.01 M $CaCl_2$) extraction (Se_{Sol}) predicted bioavailability reasonably well (r=0.84) (Fig. 4a). However, this produced a slight overestimation of bioavailability for some of the freshly spiked soils with a high sorption capacity, such as the highly calcareous Mallala and high-OM Inman Valley soils, as identified by separate symbols on Fig. 4a. It is likely that using the Se_{Sol} fraction of such soils to predict bioavailability, especially at d 1, is not a realistic depiction of the pool of labile Se available for plant uptake over their growing period (6 weeks) prior to the first sampling point (Se_{Plant} at d 1), as significant ageing will have already occurred. Nevertheless, the exclusion of the d 1 data Se_{Plant} concentrations did not significantly affect the correlation between Se_{Plant} and Se_{Sol} (r=0.84) (data not shown). These results demonstrated that, although reasonably reliable, the use of chemical extractions to predict Se bioavailability in highly sorptive soils should be viewed with caution.

Table 5: Concentrations of Se in plants grown in soils that were aged with Se for 1, 30, 60, 90 and 300 days. Results show averages ± standard errors (n=4). An ageing factor (AF_{plant}) was calculated as the ratio of Se_{Plant} at the beginning (d 1) and end of the experiment (d 300).

Soils	Se concentrations in plants (mg kg^{-1}) grown in soils aged with Se for *t* days					AF_{plant}
	1	*30*	*60*	*90*	*300*	
Mt Compass	142 ± 16.0	127 ± 16	82.3 ± 4.5	78.9 ± 0.6	56.9 ± 1.0	2.49
Inman Valley	30.8 ± 3.9	12.7 ± 1.3	13.8 ± 1.5	9.84 ± 0.7	6.06 ± 0.2	5.08
Charleston	1.33 ± 0.2	22.0 ± 1.2	9.6 ± 0.8	8.4 ± 0.2	5.3 ± 0.2	1.83[a]
Kingaroy	10.9 ± 0.6	12.0 ± 5.5	11.2 ± 0.5	10.0 ± 0.4	9.05 ± 1.0	1.20
Balaklava	92.5 ± 14.0	87.0 ± 3.1	29.8 ± 2.2	3.47 ± 0.2	0.27 ± 0.1	338
Black Point	85.9 ± 5.9	4.85 ± 0.7	29.1 ± 4.2	11.3 ± 1.0	0.31 ± 0.0	280
Mallala	37.0 ± 2.7	2.94 ± 0.2	0.80 ± 0.02	0.76 ± 0.1	0.36 ± 0.0	102
Monarto	78.7 ± 7.8	75.0 ± 9.0	65.6 ± 4.5	65.2 ± 4.5	59.7 ± 2.9	1.32

[a]: The AF_{plant} for Charleston was calculated as the ratio between Se_{Plant} at d 30 and d 300. The Se_{Plant} concentration at d 1 was unusually low, which potentially stemmed from the very poor growth of plants at that stage, presumably due to external factors such as low nutrient availability despite basal fertilisation.

The Se_{Ads} fraction should be considered a potential source of plant-available Se in the soil, as Se_{Ads} can desorb to replenish the soluble Se pool in soil solution in response to depletion (Dhillon et al., 2005). Effectively, a high correlation (r=0.83) between KH_2PO_4-extractable soil Se and plant Se concentration was observed in studies by Zhao et al. (2005). However, in our study, correlating the 'plant-available' Se (Se_{Sol} + Se_{Ads}) with Se_{Plant} (r=0.69) did not improve the prediction for bioavailability over Se_{Sol} alone (r=0.84) (Fig. 4b). This was probably because the adsorbed Se fraction in most soils, apart from the Oxisol, was minimal (< 10% of the total soil Se). Our results were in agreement with those of Dhillon et al. (2005), in which the chemical extraction of Se from seleniferous soils (> 0.5 mg Se kg^{-1}) by either hot water or potassium chloride (KCl), was more effective (r=0.70) in predicting bioavailability in wheat than its extraction with KH_2PO_4. Hence, the suitability of a chemical extractant in extracting plant-available Se appears to depend on the type and nature of the soil (Dhillon et al., 2005).

Ageing factors were derived based on both the plant concentrations (AF_{plant}) and chemical-extraction data (AF_{extr}). They were calculated as the ratio of the Se concentration in the plant or in the $CaCl_2$ extract in freshly spiked soil and that aged for 300 d (Se_{Plant} 1 d:300 d or Se_{Sol} 1 d:300 d). The ageing was much more pronounced for the three soils with $CaCO_3$ > 2%, in which the plant Se concentrations decreased > 100-fold with ageing, than in the other soils (Table 5). For the non-calcareous soils, the greatest ageing factor was in the Inman Valley soil. Since Inman Valley had the highest SOC content (Table 1), this suggests that Se incorporation into OM was the primary ageing mechanism in non-calcareous soils. The biological ageing factor (AF_{plant}) was strongly correlated with the chemical extraction-based ageing factor (AF_{extr}) (r=0.93) (Fig. 5), hence confirming the possible use of chemical extraction of soils with $CaCl_2$ to estimate Se bioavailability.

5 Conclusions

The availability of added Se^{VI} in soil decreased with ageing time, which was observed both in chemically-extracted Se_{Sol} fractions and concentrations of Se in plants. However, the rate of decrease varied significantly among the different soils studied. The change in Se solubility as a function of time was best represented by a reversible first order equation model in virtually all the soils. Ageing was most pronounced in calcareous soils, with the predicted soluble fraction at equilibrium (f_{eq}) close to zero. The rate of Se ageing in soils increased with an increasing $CaCO_3$ content; in non-calcareous soils, ageing was less pronounced. Chemical extraction of soils with dilute $CaCl_2$ was effective in predicting bioavailability, although slight overestimation of Se_{Plant} from highly sorptive soils such as Mallala and Inman Valley, where solubility significantly dropped in the first 30 d, was observed.

Even though the fit of the data to the RFO model did not reveal the processes driving Se ageing in the different soils, it showed consistency between chemical and biological assays in estimating the kinetics of ageing processes and the change in bioavailability of Se over time. With calibration, the model could potentially be used to estimate the biofortification requirements of crops grown on different soil types. For example, accounting for the progressive Se fixation capacity of different soils, it may be useful to apply Se fertilisers in small doses more frequently to optimise efficiency of use and minimise environmental hazard.

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7 References

Banuelos, G., Schrale, G., 1989. Plants that remove selenium from soils. Calif. Agric. (3), 19-20.

Barrow, N.J., 1998. Effects of time and temperature on the sorption of cadmium, zinc, cobalt, and nickel by a soil. Aust. J. Soil Res. 36, 941-950.

Barrow, N.J., Whelan, B.R., 1989. Testing a mechanistic model. VIII. The effects of time and temperature of incubation on the sorption and subsequent desorption of selenite and selenate by a soil. J. Soil Sci. 40(1), 29-37.

Boostani, H.R., Najafi-Ghiri, M., Amin, H., Mirsoleimani, A., 2019. Zinc desorption kinetics from some calcareous soils of orange (*Citrus sinensis* L.) orchards, southern Iran. J. Soil Sci. Plant Nutr. 65(1), 20-27.

Bowden, J.W., Nagarajah, S., Barrow, N.J., Posner, A.M., Quirk, J.P., 1980. Describing the adsorption of phosphate, citrate and selenite on a variable-charge mineral surface. Aust. J. Soil Res. 18(1), 49-60.

Buekers, J., 2007. Fixation of cadmium, copper, nickel and zinc in soil: kinetics, mechanisms and its effect on metal bioavailability, KU Leuven.

Buekers, J., Degryse, F., Maes, A., Smolders, E., 2008. Modelling the effects of ageing on Cd, Zn, Ni and Cu solubility in soils using an assemblage model. Eur. J. Soil Sci. 59(6), 1160-1170.

Cowan, C.E., Zachara, J.M., Resch, C.T., 1990. Solution ion effects on the surface exchange of selenite on calcite. Geochim. Cosmochim. Acta 54(8), 2223-2234.

Crout, N.M.J., Tye, A.M., Zhang, H., McGrath, S.P., Young, S.D., 2006. Kinetics of metal fixation in soils: measurement and modeling by isotopic dilution. Environ. Toxicol. Chem. 25(3), 659-663.

Degryse, F., Smolders, E., Parker, D.R., 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications - a review. Eur. J. Soil Sci. 60(4), 590.

Dhillon, K.S., Dhillon, S.K., 1999. Adsorption–desorption reactions of selenium in some soils of India. Geoderma 93(1), 19-31.

Dhillon, K.S., Rani, N., Dhillon, S.K., 2005. Evaluation of different extractants for the estimation of bioavailable selenium in seleniferous soils of Northwest India. Aust. J. Soil Res. 43(5), 639-645.

Dinh, Q.T., Wang, M.Z., Tran, T., Zhou, F., Wang, D., Zhai, H., Peng, Q., Xue, M., Du, Z., Bañuelos, G.S., Lin, Z., Liang, D., 2019. Bioavailability of selenium in soil-plant system and a regulatory approach. Crit. Rev. Environ. Sci. Technol. 49(6), 443-517.

Dodge, Y., 2008. The Concise Encyclopedia of Statistics. Springer 1st. ed. ed. Springer New York, New York, NY.

Elrashidi, M.A., Adriano, D.C., Workman, S.M., Lindsay, W.L., 1987. Chemical equilibria of selenium in soils: a theoretical development. Soil Sci. 144(2), 141-152.

Frost, R.R., Griffin, R.A., 1977. Effect of pH on adsorption of arsenic and selenium from landfill leachate by clay minerals. Soil Sci. Soc. Am. J. 41(1), 53-57.

Gissel-Nielsen, G., Hamdy, A.A., 1977. Leaching of added selenium in soils low in native selenium. Z. Pflanzenernaehr. Bodenkd. 140(2), 193-198.

Goldberg, S., 2014. Modeling selenate adsorption behavior on oxides, clay minerals, and soils using the triple layer model. Soil Sci. 179(12), 568.

Goldberg, S., Glaubig, R.A., 1988. Anion sorption on a calcareous, montmorillonitic soil: selenium. Soil Sci. Soc. Am. J. 52(4), 954-958.

Gupta, U.C., Gupta, S.C., 2000. Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. Commun. Soil Sci. Plant Anal. 31(11-14), 1791-1807.

Hassan, S.W., 2011. Iodine dynamics in soil, University of Nottingham, Nottingham, United Kingdom.

Haug, A., Graham, R.D., Christophersen, O.A., Lyons, G.H., 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. Microb. Ecol. Health Dis. 19(4), 209-228.

Heming, S.D., 2007. Phosphorus balances for arable soils in Southern England 1986–1999. Soil Use Manage. 23(2), 162-170.

Hingston, F.J., Atkinson, R.J., Posner, A.M., Quirk, J.P., 1967. Specific adsorption of anions. Nature 215(5109), 1459-1461.

Howard, P.J.A., 1965. The carbon-organic matter factor in various soil types. Oikos 15(2), 229-236.

Islas-Espinoza, M., Solís-Mejía, L., Esteller, M., 2014. Phosphorus release kinetics in a soil amended with biosolids and vermicompost. Environ. Earth Sci. 71(3), 1441-1451.

Janik, L.J., Soriano-Disla, J., Forrester, S.T., McLaughlin, M.J., 2016. Effects of soil composition and preparation on the prediction of particle size distribution using mid-infrared spectroscopy and partial least-squares regression. Soil Res. 54(8), 889-904.

Jones, G., Belling, G., 1967. The movement of copper, molybdenum, and selenium in soils as indicated by radioactive isotopes. Aust. J. Agric. Res. 18(5), 733.

Keskinen, R., Ekholm, P., Yli-Halla, M., Hartikainen, H., 2009. Efficiency of different methods in extracting selenium from agricultural soils of Finland. Geoderma 153(1), 87-93.

Lamble, G.M., Lee, J.F., Staudt, W.J., Reeder, R.J., 1995. Structural studies of selenate incorporation into calcite crystals. Physica B. 208(C), 589-590.

Li, J., Peng, Q., Liang, D., Liang, S., Chen, J.S., Sun, H., Li, S., Lei, P., 2016. Effects of aging on the fraction distribution and bioavailability of selenium in three different soils. Chemosphere 144, 2351-2359.

Li, Z., Liang, D., Peng, Q., Cui, Z., Huang, J., Lin, Z., 2017. Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: A review. Geoderma 295, 69-79.

Martin, A.E., Reeve, R., 1955. A rapid nanometric method for determining soil carbonate Soil Sci. 79(3), 187-198.

Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. Commun. Soil Sci. Plant Anal. 17(18), 1499-1511.

Mathers, A., Young, S.D., McGrath, S., Zhao, F., Crout, N., Bailey, E.H., 2017. Determining the fate of selenium in wheat biofortification: an isotopically labelled field trial study. Plant and Soil 420(1), 61-77.

Mayland, H.F., Gough, L.P., Stewart, K.C., 1991. Selenium mobility in soils and its absorption, translocation, and metabolism in plants, USGS Circular No. 1064, USA.

Muller, J., Abdelouas, A., Ribet, S., Grambow, B., 2012. Sorption of selenite in a multi-component system using the “dialysis membrane” method. Appl. Geochem. 27(12), 2524-2532.

Neal, R.H., Sposito, G., Holtzclaw, K.M., Traina, S.J., 1987. Selenite adsorption on alluvial soils. I. soil composition and pH effects. Soil Sci. Soc. Am. J. (5), 1161-1165.

Peak, D., 2006. Adsorption mechanisms of selenium oxyanions at the aluminum oxide/water interface. J. Colloid Interface Sci. 303(2), 337-345.

Peak, D., Sparks, D., 2002. Mechanisms of selenate adsorption on iron oxides and hydroxides. Environ. Sci. Technol. 36.

Peng, Q., Li, J., Wang, D., Wei, T., Chen, C., Liang, D., 2019. Effects of ageing on bioavailability of selenium in soils assessed by diffusive gradients in thin-films and sequential extraction. Plant and Soil 436(1-2), 159-171.

Qin, H.-B., Zhu, J.-M., Su, H., 2012. Selenium fractions in organic matter from Se-rich soils and weathered stone coal in selenosis areas of China. Chemosphere 86(6), 626-633.

Rayman, M.P., 2012. Selenium and human health. Lancet 379(9822), 1256-1268.

Rayment, G.E., Higginson, F.R., 1992. Australian laboratory handbook of soil and water chemical methods. Inkata Press, Melbourne.

Rayment, G.E., Lyons, D.J., 2011. Soil chemical methods: Australasia. CSIRO Pub., Collingwood, Vic.

Reeder, R.J., Lamble, G.M., Lee, J.F., Staudt, W.J., 1994. Mechanism of SeO42− substitution in calcite: An XAFS study. Geochim. Cosmochim. Acta 58(24), 5639-5646.

Renard, F., Montes-Hernandez, G., Ruiz-Agudo, E., Putnis, C.V., 2013. Selenium incorporation into calcite and its effect on crystal growth: An atomic force microscopy study. Chem. Geol. 340(C), 151-161.

Shock, E.L., Helgeson, H.C., 1988. Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: Correlation algorithms for ionic species and equation of state predictions to 5 kb and 1000°C. Geochim. Cosmochim. Acta 52(8), 2009-2036.

Shrivastava, A., Gupta, V., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron. Young Sci. 2(1), 21-25.

Singh, M., Singh, N., Relan, P.S., 1981. Adsorption and desorption of selenite and selenate selenium on different soils [Normal, calcareous, high organic carbon saline, and alkali]. Soil Sci. 132(2), 134-141.

Smažíková, P., Praus, L., Száková, J., Tremlová, J., Hanč, A., Tlustoš, P., 2017. The effect of organic matter rich amendments on selenium mobility in soil. Pedosphere.

Sparks, D.L., 2003. Environmental soil chemistry. 2nd ed. ed. Academic Press, Amsterdam.

Supriatin, S., Weng, L., Comans, R., 2016. Selenium-rich dissolved organic matter determines selenium uptake in wheat grown on low-selenium arable land soils. Plant and Soil 408(1), 73-94.

Wang, D., Zhou, F., Yang, W., Peng, Q., Man, N., Liang, D., 2017. Selenate redistribution during aging in different Chinese soils and the dominant influential factors. Chemosphere 182, 284-292.

Wang, M., Cui, Z., Xue, M., Peng, Q., Zhou, F., Wang, D., Dinh, Q.T., Liang, D., 2019. Assessing the uptake of selenium from naturally enriched soils by maize (*Zea mays* L.) using diffusive gradients in thin-films technique (DGT) and traditional extractions. Sci. Total Environ. 689, 1-9.

Wright, M.T., Parker, D.R., Amrhein, C., 2003. Critical evaluation of the ability of sequential extraction procedures to quantify discrete forms of selenium in sediments and soils. Environ. Sci. Technol. 37(20), 4709-4716.

Zhang, P., Sparks, D., 1990. Kinetics of selenate and selenite adsorption/desorption at the goethite/water interface. Environ. Sci. Technol. 24(12), 1848-1856.

Zhao, C., Ren, J., Xue, C., Lin, E., 2005. Study on the relationship between soil selenium and plant selenium uptake. Plant and Soil 277(1), 197-206.

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Effect of soil properties on time-dependent fixation (ageing) of selenate

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Highlights

- Ageing of Se was more pronounced in calcareous soils than non-calcareous ones.
- Selenium ageing was well represented by a reversible first order equation.
- Chemical extraction of soils with $CaCl_2$ was effective in predicting Se bioavailability.

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1 Abstract

Previous biofortification studies have established that the residual effect of added selenium (Se) fertilisers on second-season crops is minimal. To explore the fate of exogenous Se in soil, chemical and biological methods were employed to assess the change in Se bioavailability with time. Eight soils varying in physicochemical properties were spiked with sodium selenate (0.5 mg kg^{-1} Se) and incubated at 25°C for different periods (1, 30, 60, 90 and 300 d). At the end of the incubation, soil Se was fractionated by a sequential extraction procedure into 'soluble', 'adsorbed' and 'organically-bound' Se fractions. Simultaneously, wheat was grown in the Se-aged soils, under controlled conditions for six weeks, and uptake was determined. A general decrease in Se solubility over time was observed, but the rate of decrease varied depending on soil type. A reversible first order model fitted the Se ageing kinetics well, except in an Oxisol. The most pronounced ageing was observed in calcareous soils. Concentrations of Se in the shoots of wheat grown in freshly spiked soils ranged from 71.8 ± 17.5 mg kg^{-1} in calcareous soils to 110 ± 31.6 mg kg^{-1} in non-calcareous, low-OM soils. With ageing, shoot Se concentrations decreased to < 10 mg kg^{-1}, with the lowest concentrations (0.31 ± 0.03 mg kg^{-1}) measured in plants grown in calcareous soils. The Se concentrations in plants correlated well with the soluble Se fraction (extracted with 0.01 M $CaCl_2$). The findings of this study can be used to make more informed decisions about the rate and frequency of Se fertiliser application in agronomic biofortification programs.

Keywords: selenate; ageing; chemical extractions; pot trial; modelling

Highlights

- Ageing of Se was more pronounced in calcareous soils than non-calcareous ones.
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2 Introduction

Selenium (Se) is an essential nutrient required for proper functioning of the human immune system. In the last four decades, a decline in human dietary Se levels has triggered research into ways by which Se consumption can be increased in a sustainable manner (Haug et al., 2007). Applying Se fertilisers to staple crops, a process known as biofortification, is highly effective in increasing Se concentrations in edible crop parts, such as wheat grain, destined for human nutrition (Gupta and Gupta, 2000). However, excessive dietary intake of Se can also be detrimental to human health (Rayman, 2012). It is therefore vital to understand how applied Se is transferred within the soil-plant system and determine how factors such as soil conditions and contact time influence that transfer.

Inorganic Se exists mainly as anionic oxy-acid species in most agricultural soils, in the form of selenate (SeO_4^{2-} or Se^{VI}) and selenite (SeO_3^{2-} or Se^{IV}) (Banuelos and Schrale, 1989; Gupta and Gupta, 2000). In alkaline aerobic soils, Se^{VI} tends to predominate while in more reducing acidic conditions, Se^{IV} is usually the prevalent inorganic species (Elrashidi et al., 1987). Selenium ions have different sorptive behaviour in soil, which also results in a difference in their mobility. Anions are adsorbed onto soil geocolloidal phases by several mechanisms, including inner- and outer-sphere complexation. Outer-sphere complexes, formed by weak electrostatic forces of attraction between ions and the functional groups of soil sorbent phases, are usually reversible (Bowden et al., 1980). On the other hand, inner-sphere complexation, which occurs by covalent bonding between the ions and the functional groups, results in stronger sorption behaviour. Selenite ions are adsorbed specifically by inner-sphere complexation onto surfaces of soil components such as oxides of aluminium (Al) and iron (Fe) as well as clay mineral edges (Peak, 2006; Zhang and Sparks, 1990). Selenate, on the other hand, tends to form mostly outer-sphere complexes, which explains its lower sorption and higher mobility compared to Se^{IV} in soils (Peak and Sparks, 2002; Sparks, 2003). Soil pH is a major factor influencing Se mobility through its influence on the surface charge of minerals and the speciation of Se oxyanions. For example, greater sorption of Se^{IV} onto soil mineral phases is observed under acidic, compared to neutral/alkaline, conditions (Dhillon and Dhillon, 1999; Neal et al., 1987). Studies by Gissel-Nielsen and Hamdy (1977) also highlighted the importance of the mineral phases present on the Se sorption capacity of soils; for example, the adsorption of selenite on 2:1 aluminosilicate minerals such as montmorillonite is greater than on 1:1 minerals such as kaolinite, due to the greater surface area available for anion sorption (Frost and Griffin, 1977). The presence of soil organic matter (SOM) also influences Se bioavailability (Li et al., 2017). Depending on the type of complexes formed between Se and SOM fractions, Se is irreversibly immobilised or weakly retained (Smažíková et al., 2017). As described by Qin et al. (2012), Se is more weakly bound to fulvic acids (FA) compared to humic acids (HA). Given the greater solubility of FA, there is also the potential for FA-bound Se to dissolve in soil pore water and become bioavailable or even leach out the of root zone to nearby water bodies, potentially becoming a source of contamination to aquatic biota. Leaching of weakly-bound organic Se was posited as an explanation for a sudden surge in human selenosis cases in a high-Se area in China (Qin et al., 2012).

The total concentration of Se in soil is not a good indicator of its bioavailability (Gupta and Gupta, 2000). Instead, chemical extraction methods (single or sequential) can be employed to fractionate Se in soils and may provide a better estimate of Se bioavailability. ‘Soluble’ Se is usually extracted using hot water or a dilute salt solution based on anion exchange (Wright et al., 2003), while ‘adsorbed’ Se can be extracted using a phosphate solution through ligand exchange; phosphate adsorbs more strongly than selenite on soil surfaces (Hingston et al., 1967; Keskinen et al., 2009). The organically-bound Se fraction is rather difficult to separate from inorganic Se species, but alkaline reagents such as sodium hydroxide (NaOH) and tetramethylammonium hydroxide (TMAH) solubilise organic matter (OM) and release OM-bound Se (Hingston et al., 1967). An advantage of TMAH over inorganic extractants such as NaOH is that pH can be increased without concurrently increasing the salt concentration of the solution, which can limit the sensitivity of inductively coupled plasma mass spectrometers (ICP-MS) typically used for Se analysis (Hassan, 2011). Although laborious and operationally defined, sequential extraction procedures (SEPs) are useful to fractionate analytes and can be used to assess changes in extractability over time.

When metals are added to the soil, their solubility decreases towards an asymptotic equilibrium – as a result of fixation reactions in the soil (Buekers, 2007). The fixation mechanisms of trace metals such as Pb, Zn, Cu and Co have been explored thoroughly (Barrow, 1998; Degryse et al., 2009) while those of Se are still relatively poorly understood. 'Fixation' hereby refers to the immobilisation process of Se that may occur progressively for days and years following initial rapid (hours) adsorption of Se in soil (Buekers, 2007); ageing refers to the time-dependent process of Se fixation in the soil. Studies about the fixation behaviour of Se showed that the chemical speciation of the anions influences their retention capacity in the soil (Barrow and Whelan, 1989): Se^{IV} is initially adsorbed and is then further retained, possibly by diffusive penetration into soil particles such as Fe oxides. This suggests that Se^{IV} continues to react with the soil for a very long time, with little desorption, and hence become increasingly unavailable to plants. Selenate, on the other hand, has been found to remain more bioavailable in soils in the short term, especially in neutral to alkaline soils, as it is weakly adsorbed onto soil particles. However with time, Se^{VI} may be reduced to lower valence state species such as Se^{IV}, Se^0 and Se^{2-}, leading to further adsorption of the reduced species onto surfaces such as carbonate and Fe/Mn oxides or incorporation into humus. This effect is slower in neutral to alkaline soils, compared to acidic soils with high Fe/Mn oxides and OM content, where faster reduction of Se^{VI} to Se^{IV} occurred (Wang et al., 2017).

Using sequential chemical extractions to partition Se in Se^{VI}-spiked soils over a period of a year, Wang et al. (2017) showed that the rate of Se ageing is primarily dictated by soil pH, Fe and Al oxides and SOM. More recently, Wang et al. (2019) and Peng et al. (2019) used the diffusive gradient thin (DGT) film technique to assess the change in bioavailable Se with time. The DGT technique can mimic the dynamic movement of Se in a soil-plant system, including the re-supply of the solution phase with adsorbed Se, but can be quite costly and labour-intensive. To validate the effectiveness of chemical extraction methods in predicting bioavailability in a cost-effective manner, biological assays need to be undertaken simultaneously. Li et al. (2016) used a combined approach of sequential extractions and a crop trial to assess the ageing of Se in soil. However, their study only comprised three soils and so could not relate Se ageing to soil characteristics.

Our study addressed this gap by combining chemical, biological and predictive modelling techniques to gain a holistic understanding of the fate of added selenate fertiliser in eight soils with contrasting soil properties over a period of time (300 d) which exceeded a typical growing season. The main soil properties driving the ageing of the added Se were determined. Such information will be crucial in assessing the residual value of Se^{VI} fertilisers in different soil systems, which could be used to make more informed decisions about Se fertiliser application rates in biofortification programs.

3 Materials and Methods

3.1 Soils

Eight Australian soils were chosen to represent a range of physicochemical properties that were likely to affect Se dynamics. The soils were air-dried, sieved to < 2 mm and homogenised prior to the experimental setup. The physical and chemical properties of the soils are listed in Table 1. Briefly, soil pH and electrical conductivity (EC) were determined in a 1:5 soil-to-water suspension; the effective cation exchange capacity (ECEC) was determined by the ammonium acetate method in a 1:10 soil-to-solution ratio at pH 7 (Rayment and Higginson, 1992); the $CaCO_3$ content was determined by a manometric procedure (Martin and Reeve, 1955); total organic carbon (C) (TOC) was quantified by a dry combustion method (Matejovic, 1997); the oxalate Al and Fe contents were determined according to Rayment and Higginson (1992); the textural classification of the soils was determined using a mid-infrared spectroscopy method (Janik et al., 2016); available phosphorus (P) and sulphur (S) in the soil were measured after extraction with sodium bicarbonate and potassium chloride solutions respectively (Rayment and Lyons, 2011).

Table 1: The physicochemical characteristics of the soils used in this study. ‘b.d’ was used to denote concentrations that were below analytical detection limits.

Soils	pH	EC	ECEC	$CaCO_3$	Org C	Oxal Al	Oxal Fe	Clay	Sand	Available P	Available S	Total Se
	water	dS m^{-1}	c.mol kg^{-1}	%	%	g kg^{-1}	g kg^{-1}	%	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Mt Compass	5.4	0.01	1.7	b.d	0.2	0.1	0.1	1.0	98	5	3	0.02
Inman Valley	5.6	0.14	16.0	b.d	5.7	1.4	5.2	26.0	41	41	19	0.18
Charleston	5.8	0.09	7.8	b.d	5.1	0.4	4.6	3.6	85	190	14	0.23
Kingaroy	6.8	0.09	8.3	b.d	1.9	2.5	3.7	52.0	20	10	61	0.13
Balaklava	8.0	0.17	27.0	3.1	1.7	1.2	0.8	12.0	46	35	13	0.10
Black Point	8.0	0.12	15.9	2.7	1.1	1.0	0.8	15.0	70	5	10	0.10
Mallala	8.2	0.13	25.9	11.0	0.9	1.2	0.9	13.0	62	16	9	0.14
Monarto	8.9	0.09	5.1	1.1	0.4	0.3	0.2	2.6	95	5	5	0.05

3.2 Soil spiking and incubation

All soils were spiked with 0.5 mg Se kg^{-1} in the form of sodium selenate (Na_2SeO_4). After spiking, the soils were watered to 60% water holding capacity (WHC) and mixed to ensure homogeneity. They were left to equilibrate overnight and then split into four replicates of 500 g in plastic containers. The soils were incubated under aerobic conditions at 25°C. . During the incubation period, soils were watered regularly to maintain 60% WHC. Control soils (no added Se) were also included. The soils were incubated for 1, 30, 60, 90 and 300 d prior to extraction and plant growth assays.

3.3 Soil extractions

After incubation, the soils were dried in an oven at 40°C for 72 hours then ground to a homogenous fine powder prior to analytical procedures. Sequential extraction was carried out to determine the partitioning across different fractions, namely: soluble Se (Se_{Sol}) extracted with 0.01 M calcium chloride ($CaCl_2$), adsorbed Se (Se_{Ads}) extracted with 0.016 M potassium dihydrogen phosphate (KH_2PO_4) and organically-bound Se (Se_{Org}) extracted using 10% TMAH, adapted from methods developed by Wright et al. (2003), Supriatin et al. (2016) and Mathers et al. (2017). Briefly, 0.5 g of soil was shaken on an end-over-end shaker with 5 mL of extractant at 100 rpm overnight, followed by centrifugation at 3000 *g* for 30 minutes and finally syringe-filtration of the supernatant through 0.45 µm filters into 10-mL tubes. In between the extractions, the weight of the soil samples was recorded to account for the amount of Se carried over from the previous extraction. A separate single Se extraction by *aqua regia* digestion, adapted from the British standard method ISO 11 466, was also carried out to quantify pseudo-total Se in the soil (Se_{Tot}): 0.5 g of soil sample was cold digested overnight with concentrated hydrochloric acid (HCl), nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a ratio of 3:1:1. Subsequently, the samples were digested at 125°C for 4 hours in a closed system on a block digester. All samples were made up to 50 mL volume with Milli-Q water and stored at 5°C in polypropylene bottles pending analysis.

The method used for the single *aqua regia* extraction was verified through the use of the certified reference material NCS DC 73326 from the China National Analysis Centre for Iron and Steel (Beijing China, 2008). Results were within 10% of the certified value (1.6 ± 0.2 mg Se kg^{-1}), with a mean of 1.64 ± 0.15 mg Se kg^{-1}.

3.4 Pot experiment

In parallel to chemical extractions, wheat (*Triticum aestivum cv.* Axe) was grown in 250 g pots filled with the incubated soils, with four replicates. Prior to potting, the soils were mixed with a basal nutrient solution supplying the following nutrients (mg kg^{-1} of soil): N (80, split application), P (20), K (40), S (40), Ca (10), Mg (10), B (1.0), Cu (2.0), Mn (2.0), Mo (0.1) and Zn (2.0), and left to equilibrate overnight. After potting, two pre-germinated wheat seedlings were sown into the pots and thinned to one plant per pot two weeks later. The soils were watered regularly to maintain soil moisture at 60% WHC. Wheat was grown under controlled conditions (temperature of 23.2°C, humidity of 72% and 12 h daylight cycle) for six weeks.

The aboveground biomass of the wheat plants was hand-harvested and dried in an oven at 50°C for 72 h, or until a constant weight was achieved. The dried samples were finely ground using a laboratory-grade grinder and stored dry prior to total Se analysis. The plant Se concentration (Se_{Plant}) was determined after acid digestion:0.25 g of plant sample (four replicates) was weighed into 50-mL digestion tubes (Axygen, Thermo Fisher Scientific, New York) and left overnight in 2 mL of HNO_3 acid and 0.5 mL of H_2O_2 to predigest. The samples were then heated to 80°C for 45 min, followed by 125°C for 160 min on a block digester. After acid digestion, the samples were cooled for 30 min then made to 20 mL volume using ultrapure Milli-Q water (18.2 MΩ cm).

3.5 Sample analysis

Concentrations of total Se in soil and plant samples were determined using an inductively coupled plasma – optical emission spectrometer, ICP-OES (Optima 4300, Perkin Elmer) fitted with a continuous flow hydride generator (Hyd-ICPOES). Since only Se^{IV} is reduced to a hydride, a pre-reduction step of Se^{VI} to Se^{IV} was carried out: 3 mL of the different Se extracts ($CaCl_2$, KH_2PO_4 and TMAH) were heated with an equal volume of HCl at 90°C for 30 minutes, prior to analysis. All standards and blanks were subjected to the same treatment before injection into the Hyd-ICPOES instrument.

For data quality control, detection limits were calculated for each extraction method as three times the standard deviation of the blank measurements (Shrivastava and Gupta, 2011). Concentrations of Se above the highest calibration standard were re-analysed after a 10- or 100-fold dilution of the sample with Milli-Q water. The detection limits for Se were: 0.19, 0.15, and 0.18 $\mu g\ L^{-1}$ for $CaCl_2$, KH_2PO_4, TMAH extractions, respectively. Due to the consistently low recovery of the Se_{Org} fraction by this method, a separate single TMAH extraction, using the same protocol as above, was repeated on all soil samples and the extracts were analysed for total Se concentrations by ICP-MS (Thermo-Fisher iCAP-Q) with H_2 gas added to the collision cell at a flow rate of 4 $mL\ min^{-1}$. The Se_{Org} fraction in the present study was therefore calculated as the difference between total Se extracted by the single separate TMAH extraction (Se_{TMAH}) and the sum of soluble and adsorbed Se (Se_{Sol} + Se_{Ads}).

3.6 Kinetics models

Several kinetic models were tested for their ability to represent the change in Se bioavailability, here represented by the Se_{sol} fraction in selenate-spiked soils, with time (Table 2).

Table 2: Kinetics models for Se ageing in different soils (Boostani et al., 2019; Islas-Espinoza et al., 2014; Li et al., 2016).

Models	Equations	Parameters
Reversible First Order	Eq.1	See text
Second order	$\frac{1}{Se_t} = \frac{1}{Se_{eq}} + k_2 t$	Se_t is the concentration of Se_{Sol} (mg kg^{-1}) at specific ageing time, t (days); Se_{eq} is the concentration of Se_{Sol} at equilibrium (mg kg^{-1}); k_2 is the second-order rate constant
Power function	$\ln Se_t = \ln a + b \ln t$	a and b are both constants
Elovich equation	$Se_t = a + b \ln t$	
Parabolic diffusion	$\frac{Se_t}{Se_{eq}} = a + b\sqrt{t}$	

The most comprehensive model, the Reversible First Order (RFO) – Eq. 1 – was adapted from Buekers et al. (2008) and Crout et al. (2006) as follows:

$$f = f_{eq} + \left(f_0 - f_{eq}\right) \times e^{-t/T_c} \qquad \text{Eq. 1}$$

where f, f_0 and f_{eq} are the fractions of added Se that are soluble at time t, time 0 and at equilibrium, respectively, and T_c is the response time of the reaction (d).

All model parameters were optimised by minimising the residual standard deviations (RSD) (Eq. 2). The goodness-of-fit of the models to the selenate ageing process was assessed using a minimal RSD and the Pearson's correlation coefficients (r) generated from correlating the measured and modelled data. The RSD was calculated as follows:

$$RSD = \sqrt{\frac{\Sigma\left(Se_{meas} - Se_{model}\right)^2}{n - x}} \qquad \text{Eq. 2}$$

where Se_{meas} and Se_{model} were the measured and modelled values of $\mathrm{Se_{Sol}}$ (mg kg^{-1}), respectively; n is the number of data points, and x is the number of model parameters.

3.7 Statistical analyses

The changes in Se concentrations in various soil fractions over time were assessed for statistical significance using a repeated measures Analysis of Variance (ANOVA) with Greenhouse-Greisser correction. A Bonferroni Post Hoc test was carried out to determine the time points at which Se solubility in the soils changed significantly.

Eleven soil properties (Table 1) were used to assess the main drivers of Se ageing, represented by the fraction of Se that was soluble in the soil at equilibrium (f_{eq}), in a stepwise linear regression. The data was assessed for multicollinearity using a variance inflation factor (VIF) check. No correlation was observed between the properties (VIF < 2.0) (Dodge, 2008).

Using a simple correlation analysis, the effectiveness of chemical extraction methods to predict Se bioavailability (plant Se concentrations) was assessed. The statistical analyses were conducted in SPSS (IBM SPSS Statistics for Windows, Version 24.0., IBM Corp, Armonk, New York). The parameters of the different kinetics models were fitted to the data using the Microsoft Excel Solver function.

4 Results and Discussion

4.1 Change in soil Se fractions with ageing

The background concentrations of total Se in the soils used in this study ranged from 0.02 – 0.23 mg kg^{-1}, with the highest concentrations observed in soils with high organic matter content, such as Charleston and Inman Valley (9-10% OM, assuming a conversion factor of 1.72 from organic C) (Howard, 1965) (Table 1). Total Se concentration of the aged soils averaged 0.50 ± 0.01 mg kg^{-1}, which was 99 ± 1.6% recovery of the added Se (Se_{Add}) after correction for background Se in soil. This suggests that limited losses of Se by volatilisation occurred during the incubation period.

The solubility of Se generally decreased over time, but at various rates depending on the type of soil (Fig. 1). Sandy soils such as Monarto and Mt Compass showed no statistically significant change ($P > 0.05$) in Se_{Sol} over 300 d, as the % Se_{Add} that was soluble decreased from 96% to 71% in Monarto and from 103% to 68% in Mt Compass. For the other soils, a rapid initial decrease in Se_{Sol} was observed, followed by a more gradual partitioning into non-labile pools. The most rapid decrease in Se_{Sol} occurred in Kingaroy, where the majority of the Se_{Add} (> 50%) was not extractable by $CaCl_2$ within the first 24 h. The Kingaroy soil is rich in clay (52%) and Al and Fe oxides (2.54 and 3.69 g kg^{-1} respectively), explaining its high Se retention capacity. The second fastest decrease in Se_{Sol} was observed in the alkaline calcareous Mallala soil (11% $CaCO_3$), as solubility decreased by 95% within the first 30 d, followed by a more gradual decrease. These results compare well with those in studies by Singh et al. (1981), who showed greater Se retention in calcareous soils compared to non-calcareous, saline and alkaline soils.

The Se_{Ads} fraction showed a similar pattern of distribution in most soils (Fig. 1). A slight increase in Se_{Ads} was observed for the first 60 d, followed by a decrease and stabilisation over a longer-term. Except for the Kingaroy soil, Se_{Ads} was small at d 1 (7 ± 0.88% of Se_{Add}), increased to 16 ± 2.35% at d 30 and gradually decreased to 6 ± 0.95% at d 300, showing that Se_{Ads} was not the dominant fraction in those soils. This trend suggests that Se was immobilised by processes which retained the Se more strongly than surface adsorption. Comparatively, Kingaroy was the only soil where Se_{Ads} was higher than Se_{Sol} at every ageing time, which suggests that specific adsorption onto mineral soil fractions was the primary mechanism responsible for the loss of solubility in the Oxisol (Fig. 1). A similarly high ratio of Se_{Ads}:Se_{Sol} was observed in soils with comparable mineral oxides contents to Kingaroy in a study by Li et al. (2016). This highlights the efficacy of active sites such as those of Al and Fe oxides as well as clay minerals to rapidly adsorb Se, whether supplied as soluble Se^{VI} or Se^{IV}.

At d 1, Se_{Org} averaged 0.17 ± 0.05 mg kg^{-1} in most soils, which gradually increased with ageing time (Fig. 1). High-OM soils such as Inman Valley and Charleston were expected to have higher Se_{Org} fractions compared to others; however, Se_{Org} was the greatest in the alkaline calcareous soils Mallala, Black Point and Balaklava, suggesting that TMAH did not specifically solubilise organically-bound Se but also dissolved Se bound to carbonates. The determination of organically-associated Se often requires the use of highly alkaline reagents, such as NaOH, or oxidising agents such as sodium hypochlorite (NaOCl) and potassium persulfate ($K_2S_2O_8$). However, these reagents can lead to an overestimate of the organically-bound fraction if they release Se bound to other fractions in the soil. For example, Wright et al. (2003) found that the NaOCl extraction of sediments resulted in overestimation of the Se_{Org} fraction, as the extract was also oxidising/solubilising metal selenides such as iron selenide (FeSe). Moreover, if the phosphate extraction (for Se_{Ads}) did not strip off all surface-bound Se^{IV}, the TMAH extraction would probably desorb the remaining Se^{IV} due to surface charge and ligand exchange mechanisms. Again, this could lead to an overestimation of the Se_{Org} fraction.

4.2 Kinetics models for Se ageing in soils

For all soils, except Kingaroy, the best fit of the data to the selenate ageing process was given by the reversible first order (RFO) equation model. Kingaroy was best modelled by a Power Function (Table 3). Given that the RFO model gave the best fit for the majority of the soils, it will be the only one discussed further.

Table 3: Residual standard deviation (RSD) and Pearson's correlation coefficients (r) of kinetics models for change in soluble Se fraction over time in different soils. The numbers highlighted in bold show the best goodness-of-fit between modelled and measured Se_{Sol} values.

Kinetic models	Mt Compass		Inman Valley		Charleston		Kingaroy		Balaklava		Black Point		Mallala		Monarto	
	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r	RSD	r
Reversible first order	**0.04**	**0.88**	**0.01**	**0.99**	**0.08**	**0.91**	0.11	0.00	**0.05**	**0.99**	**0.04**	**0.94**	**0.00**	**1.00**	**0.01**	**0.98**
Second order	0.04	0.80	0.06	0.73	0.10	0.75	0.09	0.00	0.06	0.95	0.14	0.73	0.01	1.00	0.01	0.97
Power Function	0.06	0.00	0.02	0.98	0.22	0.79	**0.08**	**0.55**	0.10	0.88	0.12	0.77	0.01	1.00	0.04	0.45
Elovich	0.06	0.00	0.01	0.98	0.08	0.79	0.09	0.00	0.07	0.94	0.08	0.94	0.08	0.93	0.04	0.57
Parabolic Diffusion	0.06	0.67	0.06	0.75	0.14	0.62	0.09	0.00	0.13	0.86	0.17	0.86	0.20	0.57	0.02	0.95

Table 4: The estimated model parameters derived from the reversible first order model to predict Se solubility (Se_{Sol}) over time. Kingaroy was not well represented by the model.

Soils	Soil type	f_0	f_{eq}	T_c (d^{-1})
Mallala	Calcareous	0.97	0.01	9.40
Balaklava		1.00	0.02	48.3
Black Point		0.98	0.01	110
Monarto		0.89	0.00	943
Inman Valley	Non calcareous	0.80	0.44	18.9
Charleston		0.84	0.23	56.5
Mt Compass		0.98	0.66	121
Kingaroy	Oxisol	0.25	-	-

The RFO model parameters indicate whether there was immediate sorption of Se onto soil surfaces ($f_0 < 1$), how much of the added Se remains soluble at equilibrium (f_{eq}) and the 'response time' of the ageing reaction (T_c, the time required to reach 63% of the difference between the initial and equilibrium values) (Table 4). The capacity of soils to instantly 'fix' Se ($1-f_0$) was not well correlated with Se ageing. For example, alkaline calcareous soils such as Mallala, Balaklava and Black Point, which had a low proportion of immediately-fixed Se (< 5%), showed more pronounced Se ageing (higher f_{eq}) compared to other soils (Table 4). For the calcareous soils, almost all of the added Se was predicted to be fixed at equilibrium, and this fraction increased with increasing $CaCO_3$ content of soils, following the order of Mallala ($CaCO_3$ = 11%) > Balaklava ($CaCO_3$ = 3.1%) > Black Point ($CaCO_3$ = 2.7%) > Monarto ($CaCO_3$ = 1.1%) (Fig. 2).

Low instantaneous immobilisation of added Se in the calcareous soils suggest that more Se remained bioavailable shortly after addition probably because the alkaline, aerobic soil conditions favour the predominance of mobile Se^{VI} ions (Gupta and Gupta, 2000).

However, over time, more pronounced Se ageing occurred in the calcareous soils (Fig. 2), pointing to a strong binding mechanism, possibly by ion exchange with CO_3^{2-} on calcite surfaces (Goldberg and Glaubig, 1988). The non-calcareous soils showed relatively less pronounced ageing of Se (Table 4), and the rate of Se fixation in high-OM soils such as Inman Valley and Charleston (T_c of 19 and 57 d) was greater than that in low-OM soils such as Mt Compass and Monarto (T_c of 120 and 942 d respectively). Despite the low $CaCO_3$ content (1.1%) of Monarto, its f_{eq} was predicted to be 0 (Table 4), which suggests that Se fixation in Monarto would occur at a very slow rate over a long period of time (estimated 942 d). Given that the ageing period for this study was 300 d, it was not possible to verify this prediction. However, for all the other soils, f_{eq} was estimated to be reached within the 300 d period ($T_c < 300$; Table 4) and the good correlation observed between f_{eq} and Se_{Sol} concentrations at d 300 for those soils (r = 0.99) (data not shown) suggests that the model prediction was reliable.

While the RFO model described the pattern of ageing in most soils well, it did not indicate which mechanisms drove Se ageing. Moreover, given the lack of selectivity of certain extractants, such as TMAH, it was not possible to verify the processes that may have caused these changes. It was only possible to speculate on the basis of apparent correlations with soil properties. For example, the retention of Se onto calcite surfaces by an ion exchange mechanism may explain the more pronounced retention of Se in calcareous soils.

The Oxisol, Kingaroy, was not well represented by kinetic models because most sorption occurred in the first day (> 75% of the added Se adsorbed with 24 h) and there was little change in Se solubility afterwards. This soil was, therefore, omitted from the statistical analysis determining which soil fractions were primarily driving Se ageing.

4.3 Effect of soil properties on Se ageing

A multiple linear regression analysis between the estimated Se_{Sol} fraction at equilibrium (f_{eq}) and soil properties showed that soil pH was the main property influencing the loss in Se solubility with time, explaining 81% of the variance among the different soils ($p < 0.05$) (Fig. 3). Under aerated alkaline conditions, Se^{VI}, which is weakly retained on soil particles, is expected to predominate (Mayland et al., 1991). Hence, the more pronounced ageing at higher pH may seem surprising. However, the alkaline soils were also calcareous, and the rate of Se fixation increased with increasing $CaCO_3$ content of the soils. It seems therefore likely that retention of Se on $CaCO_3$ was the primary mechanism responsible for Se ageing in the alkaline soils.

The sorption of Se^{VI} in calcareous soils has not been studied extensively (Cowan et al., 1990; Jones and Belling, 1967; Renard et al., 2013; Singh et al., 1981). Goldberg and Glaubig (1988) showed that the retention behaviour of Se on calcite, especially Se^{IV}, was highly dependent on soil pH as sorption increased from pH 6 to 9, peaked between pH 8 and 9, and decreased at pH values > 9. Studies have used the chemical similarity of sulphate (SO_4^{2-}) and phosphate (HPO_4^{2-}, PO_4^{3-}) ions with Se^{VI} and Se^{IV}, respectively, to infer information about, and better understand, the mechanisms governing Se retention in calcareous soils (Barrow and Whelan, 1989). For example, Cowan et al. (1990) showed that, in a similar manner to HPO_4^{2-}, Se^{IV} was adsorbed on $CaCO_3$ by its exchange with CO_3^{2-}/HCO_3^- ions on anion exchange surface sites of calcite. This could be explained by the fact that both Se^{IV} (SeO_3^{2-}) and HPO_4^{2-} have similar charge, ionic size and structure (Shock and Helgeson, 1988). However, it should be noted that PO_4^{3-} was applied to the soil at a much higher concentration than Se^{IV}, which is representative of field conditions. In the field, P application is usually > 20 kg P ha^{-1}, which is > 2000 times the Se application rate (Heming, 2007). It can be argued that the precipitation of Se^{IV} ions at such low levels on calcite surfaces would be unlikely. In the case of Se^{VI}, structural studies using X-ray Absorption Fine Structure (XAFS) spectroscopy showed direct evidence of SeO_4^{2-} ions substituting for CO_3^{2-} in calcite; however, high concentrations of aqueous Se (1006 mg L^{-1}) was used in that study as well (Lamble et al., 1995). It was suggested that, although retention of Se onto calcite surfaces *via* an ion exchange mechanism seems possible, it would require substantial distortion of the CO_3^{2-} site to accommodate the large SeO_4^{2-} ions (Reeder et al., 1994). More studies are hence required to investigate the potential retention mechanism of Se onto calcite when Se is added at a realistic application rate to the soil.

In non-calcareous soils, initial (inorganic) Se retention occurs predominantly by an adsorption mechanism (Goldberg, 2014) and adsorption strength is, in turn, primarily dictated by pH and the contents of Fe/Mn and Al oxides as a result of their specific surface area and strong chelating ability (Dinh et al., 2019; Muller et al., 2012). In this study, the most rapid decline of Se_{Sol} (> 75% within 24 h) was observed in the Kingaroy soil (Fig. 1), which is an Oxisol with large Al/Fe oxide and clay contents (Table 1), suggesting that the oxides were responsible for Se sorption in this soil. In the other non-calcareous soils, ageing was most pronounced in the high-OM soils (Fig. 2), suggesting that binding onto, or incorporation into, OM played a role in the ageing process. However, it is hard to infer the type of bonding responsible for the retention of Se in OM. The chemical fractionation method employed in this study does not even differentiate between HA- and FA-bound Se; furthermore, extraction under alkaline conditions may include intact organic-Se species and inorganic Se released by alkaline hydrolysis.

4.4 Predicting availability of Se to plants

The Se concentrations of plants grown in soils aged with Se for different lengths of time over a 300 d period are shown in Table 5. The plant Se concentrations generally declined with time of incubation after Se addition, but the rate of decrease varied significantly among the different soils. Initial (t=30 d) plant Se concentrations were highest in non-calcareous, low-OM (sandy) soils (average 110 ± 31.6 mg kg^{-1}), followed by calcareous soils (average 71.9 ± 17.5 mg kg^{-1}) and were lowest in high-OM soils (average 17.3 ± 6.74 mg kg^{-1}). By the end of the ageing period, plant Se concentration decreased to < 10 mg kg^{-1} in all soils, with the exception of sandy soils (Se$_{Plant}$ average of 58.3 ± 1.40 mg kg^{-1}). The lowest concentrations at the end of the experiment were measured in plants grown in the calcareous soils Mallala, Black Point and Balaklava (average of 0.45 ± 0.03 mg kg^{-1}) (Table 5).

To determine which chemical extraction method was the best indicator of bioavailability, concentrations of Se in plants were correlated with Se$_{Sol}$ and 'plant-available' Se (Se$_{Sol}$ + Se$_{Ads}$) fractions (Fig. 4). A dilute salt (0.01 M $CaCl_2$) extraction (Se$_{Sol}$) predicted bioavailability reasonably well (r=0.84) (Fig. 4a). However, this produced a slight overestimation of bioavailability for some of the freshly spiked soils with a high sorption capacity, such as the highly calcareous Mallala and high-OM Inman Valley soils, as identified by separate symbols on Fig. 4a. It is likely that using the Se$_{Sol}$ fraction of such soils to predict bioavailability, especially at d 1, is not a realistic depiction of the pool of labile Se available for plant uptake over their growing period (6 weeks) prior to the first sampling point (Se$_{Plant}$ at d 1), as significant ageing will have already occurred. Nevertheless, the exclusion of the d 1 data Se$_{Plant}$ concentrations did not significantly affect the correlation between Se$_{Plant}$ and Se$_{Sol}$ (r=0.84) (data not shown). These results demonstrated that, although reasonably reliable, the use of chemical extractions to predict Se bioavailability in highly sorptive soils should be viewed with caution.

Table 5: Concentrations of Se in plants grown in soils that were aged with Se for 1, 30, 60, 90 and 300 days. Results show averages ± standard errors (n=4). An ageing factor (AF_{plant}) was calculated as the ratio of Se_{Plant} at the beginning (d 1) and end of the experiment (d 300).

Soils	Se concentrations in plants (mg kg^{-1}) grown in soils aged with Se for *t* days					AF_{plant}
	1	*30*	*60*	*90*	*300*	
Mt Compass	142 ± 16.0	127 ± 16	82.3 ± 4.5	78.9 ± 0.6	56.9 ± 1.0	2.49
Inman Valley	30.8 ± 3.9	12.7 ± 1.3	13.8 ± 1.5	9.84 ± 0.7	6.06 ± 0.2	5.08
Charleston	1.33 ± 0.2	22.0 ± 1.2	9.6 ± 0.8	8.4 ± 0.2	5.3 ± 0.2	1.83[a]
Kingaroy	10.9 ± 0.6	12.0 ± 5.5	11.2 ± 0.5	10.0 ± 0.4	9.05 ± 1.0	1.20
Balaklava	92.5 ± 14.0	87.0 ± 3.1	29.8 ± 2.2	3.47 ± 0.2	0.27 ± 0.1	338
Black Point	85.9 ± 5.9	4.85 ± 0.7	29.1 ± 4.2	11.3 ± 1.0	0.31 ± 0.0	280
Mallala	37.0 ± 2.7	2.94 ± 0.2	0.80 ± 0.02	0.76 ± 0.1	0.36 ± 0.0	102
Monarto	78.7 ± 7.8	75.0 ± 9.0	65.6 ± 4.5	65.2 ± 4.5	59.7 ± 2.9	1.32

[a]: The AF_{plant} for Charleston was calculated as the ratio between Se_{Plant} at d 30 and d 300. The Se_{Plant} concentration at d 1 was unusually low, which potentially stemmed from the very poor growth of plants at that stage, presumably due to external factors such as low nutrient availability despite basal fertilisation.

The Se_{Ads} fraction should be considered a potential source of plant-available Se in the soil, as Se_{Ads} can desorb to replenish the soluble Se pool in soil solution in response to depletion (Dhillon et al., 2005). Effectively, a high correlation (r=0.83) between KH_2PO_4-extractable soil Se and plant Se concentration was observed in studies by Zhao et al. (2005). However, in our study, correlating the 'plant-available' Se (Se_{Sol} + Se_{Ads}) with Se_{Plant} (r=0.69) did not improve the prediction for bioavailability over Se_{Sol} alone (r=0.84) (Fig. 4b). This was probably because the adsorbed Se fraction in most soils, apart from the Oxisol, was minimal (< 10% of the total soil Se). Our results were in agreement with those of Dhillon et al. (2005), in which the chemical extraction of Se from seleniferous soils (> 0.5 mg Se kg^{-1}) by either hot water or potassium chloride (KCl), was more effective (r=0.70) in predicting bioavailability in wheat than its extraction with KH_2PO_4. Hence, the suitability of a chemical extractant in extracting plant-available Se appears to depend on the type and nature of the soil (Dhillon et al., 2005).

Ageing factors were derived based on both the plant concentrations (AF_{plant}) and chemical-extraction data (AF_{extr}). They were calculated as the ratio of the Se concentration in the plant or in the $CaCl_2$ extract in freshly spiked soil and that aged for 300 d (Se_{Plant} 1 d:300 d or Se_{Sol} 1 d:300 d). The ageing was much more pronounced for the three soils with $CaCO_3$ > 2%, in which the plant Se concentrations decreased > 100-fold with ageing, than in the other soils (Table 5). For the non-calcareous soils, the greatest ageing factor was in the Inman Valley soil. Since Inman Valley had the highest SOC content (Table 1), this suggests that Se incorporation into OM was the primary ageing mechanism in non-calcareous soils. The biological ageing factor (AF_{plant}) was strongly correlated with the chemical extraction-based ageing factor (AF_{extr}) (r=0.93) (Fig. 5), hence confirming the possible use of chemical extraction of soils with $CaCl_2$ to estimate Se bioavailability.

5 Conclusions

The availability of added Se^{VI} in soil decreased with ageing time, which was observed both in chemically-extracted Se_{Sol} fractions and concentrations of Se in plants. However, the rate of decrease varied significantly among the different soils studied. The change in Se solubility as a function of time was best represented by a reversible first order equation model in virtually all the soils. Ageing was most pronounced in calcareous soils, with the predicted soluble fraction at equilibrium (f_{eq}) close to zero. The rate of Se ageing in soils increased with an increasing $CaCO_3$ content; in non-calcareous soils, ageing was less pronounced. Chemical extraction of soils with dilute $CaCl_2$ was effective in predicting bioavailability, although slight overestimation of Se_{Plant} from highly sorptive soils such as Mallala and Inman Valley, where solubility significantly dropped in the first 30 d, was observed.

Even though the fit of the data to the RFO model did not reveal the processes driving Se ageing in the different soils, it showed consistency between chemical and biological assays in estimating the kinetics of ageing processes and the change in bioavailability of Se over time. With calibration, the model could potentially be used to estimate the biofortification requirements of crops grown on different soil types. For example, accounting for the progressive Se fixation capacity of different soils, it may be useful to apply Se fertilisers in small doses more frequently to optimise efficiency of use and minimise environmental hazard.

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7 References

Banuelos, G., Schrale, G., 1989. Plants that remove selenium from soils. Calif. Agric. (3), 19-20.

Barrow, N.J., 1998. Effects of time and temperature on the sorption of cadmium, zinc, cobalt, and nickel by a soil. Aust. J. Soil Res. 36, 941-950.

Barrow, N.J., Whelan, B.R., 1989. Testing a mechanistic model. VIII. The effects of time and temperature of incubation on the sorption and subsequent desorption of selenite and selenate by a soil. J. Soil Sci. 40(1), 29-37.

Boostani, H.R., Najafi-Ghiri, M., Amin, H., Mirsoleimani, A., 2019. Zinc desorption kinetics from some calcareous soils of orange (*Citrus sinensis* L.) orchards, southern Iran. J. Soil Sci. Plant Nutr. 65(1), 20-27.

Bowden, J.W., Nagarajah, S., Barrow, N.J., Posner, A.M., Quirk, J.P., 1980. Describing the adsorption of phosphate, citrate and selenite on a variable-charge mineral surface. Aust. J. Soil Res. 18(1), 49-60.

Buekers, J., 2007. Fixation of cadmium, copper, nickel and zinc in soil: kinetics, mechanisms and its effect on metal bioavailability, KU Leuven.

Buekers, J., Degryse, F., Maes, A., Smolders, E., 2008. Modelling the effects of ageing on Cd, Zn, Ni and Cu solubility in soils using an assemblage model. Eur. J. Soil Sci. 59(6), 1160-1170.

Cowan, C.E., Zachara, J.M., Resch, C.T., 1990. Solution ion effects on the surface exchange of selenite on calcite. Geochim. Cosmochim. Acta 54(8), 2223-2234.

Crout, N.M.J., Tye, A.M., Zhang, H., McGrath, S.P., Young, S.D., 2006. Kinetics of metal fixation in soils: measurement and modeling by isotopic dilution. Environ. Toxicol. Chem. 25(3), 659-663.

Degryse, F., Smolders, E., Parker, D.R., 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications - a review. Eur. J. Soil Sci. 60(4), 590.

Dhillon, K.S., Dhillon, S.K., 1999. Adsorption–desorption reactions of selenium in some soils of India. Geoderma 93(1), 19-31.

Dhillon, K.S., Rani, N., Dhillon, S.K., 2005. Evaluation of different extractants for the estimation of bioavailable selenium in seleniferous soils of Northwest India. Aust. J. Soil Res. 43(5), 639-645.

Dinh, Q.T., Wang, M.Z., Tran, T., Zhou, F., Wang, D., Zhai, H., Peng, Q., Xue, M., Du, Z., Bañuelos, G.S., Lin, Z., Liang, D., 2019. Bioavailability of selenium in soil-plant system and a regulatory approach. Crit. Rev. Environ. Sci. Technol. 49(6), 443-517.

Dodge, Y., 2008. The Concise Encyclopedia of Statistics. Springer 1st. ed. ed. Springer New York, New York, NY.

Elrashidi, M.A., Adriano, D.C., Workman, S.M., Lindsay, W.L., 1987. Chemical equilibria of selenium in soils: a theoretical development. Soil Sci. 144(2), 141-152.

Frost, R.R., Griffin, R.A., 1977. Effect of pH on adsorption of arsenic and selenium from landfill leachate by clay minerals. Soil Sci. Soc. Am. J. 41(1), 53-57.

Gissel-Nielsen, G., Hamdy, A.A., 1977. Leaching of added selenium in soils low in native selenium. Z. Pflanzenernaehr. Bodenkd. 140(2), 193-198.

Goldberg, S., 2014. Modeling selenate adsorption behavior on oxides, clay minerals, and soils using the triple layer model. Soil Sci. 179(12), 568.

Goldberg, S., Glaubig, R.A., 1988. Anion sorption on a calcareous, montmorillonitic soil: selenium. Soil Sci. Soc. Am. J. 52(4), 954-958.

Gupta, U.C., Gupta, S.C., 2000. Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. Commun. Soil Sci. Plant Anal. 31(11-14), 1791-1807.

Hassan, S.W., 2011. Iodine dynamics in soil, University of Nottingham, Nottingham, United Kingdom.

Haug, A., Graham, R.D., Christophersen, O.A., Lyons, G.H., 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. Microb. Ecol. Health Dis. 19(4), 209-228.

Heming, S.D., 2007. Phosphorus balances for arable soils in Southern England 1986–1999. Soil Use Manage. 23(2), 162-170.

Hingston, F.J., Atkinson, R.J., Posner, A.M., Quirk, J.P., 1967. Specific adsorption of anions. Nature 215(5109), 1459-1461.

Howard, P.J.A., 1965. The carbon-organic matter factor in various soil types. Oikos 15(2), 229-236.

Islas-Espinoza, M., Solís-Mejía, L., Esteller, M., 2014. Phosphorus release kinetics in a soil amended with biosolids and vermicompost. Environ. Earth Sci. 71(3), 1441-1451.

Janik, L.J., Soriano-Disla, J., Forrester, S.T., McLaughlin, M.J., 2016. Effects of soil composition and preparation on the prediction of particle size distribution using mid-infrared spectroscopy and partial least-squares regression. Soil Res. 54(8), 889-904.

Jones, G., Belling, G., 1967. The movement of copper, molybdenum, and selenium in soils as indicated by radioactive isotopes. Aust. J. Agric. Res. 18(5), 733.

Keskinen, R., Ekholm, P., Yli-Halla, M., Hartikainen, H., 2009. Efficiency of different methods in extracting selenium from agricultural soils of Finland. Geoderma 153(1), 87-93.

Lamble, G.M., Lee, J.F., Staudt, W.J., Reeder, R.J., 1995. Structural studies of selenate incorporation into calcite crystals. Physica B. 208(C), 589-590.

Li, J., Peng, Q., Liang, D., Liang, S., Chen, J.S., Sun, H., Li, S., Lei, P., 2016. Effects of aging on the fraction distribution and bioavailability of selenium in three different soils. Chemosphere 144, 2351-2359.

Li, Z., Liang, D., Peng, Q., Cui, Z., Huang, J., Lin, Z., 2017. Interaction between selenium and soil organic matter and its impact on soil selenium bioavailability: A review. Geoderma 295, 69-79.

Martin, A.E., Reeve, R., 1955. A rapid nanometric method for determining soil carbonate Soil Sci. 79(3), 187-198.

Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. Commun. Soil Sci. Plant Anal. 17(18), 1499-1511.

Mathers, A., Young, S.D., McGrath, S., Zhao, F., Crout, N., Bailey, E.H., 2017. Determining the fate of selenium in wheat biofortification: an isotopically labelled field trial study. Plant and Soil 420(1), 61-77.

Mayland, H.F., Gough, L.P., Stewart, K.C., 1991. Selenium mobility in soils and its absorption, translocation, and metabolism in plants, USGS Circular No. 1064, USA.

Muller, J., Abdelouas, A., Ribet, S., Grambow, B., 2012. Sorption of selenite in a multi-component system using the “dialysis membrane” method. Appl. Geochem. 27(12), 2524-2532.

Neal, R.H., Sposito, G., Holtzclaw, K.M., Traina, S.J., 1987. Selenite adsorption on alluvial soils. I. soil composition and pH effects. Soil Sci. Soc. Am. J. (5), 1161-1165.

Peak, D., 2006. Adsorption mechanisms of selenium oxyanions at the aluminum oxide/water interface. J. Colloid Interface Sci. 303(2), 337-345.

Peak, D., Sparks, D., 2002. Mechanisms of selenate adsorption on iron oxides and hydroxides. Environ. Sci. Technol. 36.

Peng, Q., Li, J., Wang, D., Wei, T., Chen, C., Liang, D., 2019. Effects of ageing on bioavailability of selenium in soils assessed by diffusive gradients in thin-films and sequential extraction. Plant and Soil 436(1-2), 159-171.

Qin, H.-B., Zhu, J.-M., Su, H., 2012. Selenium fractions in organic matter from Se-rich soils and weathered stone coal in selenosis areas of China. Chemosphere 86(6), 626-633.

Rayman, M.P., 2012. Selenium and human health. Lancet 379(9822), 1256-1268.

Rayment, G.E., Higginson, F.R., 1992. Australian laboratory handbook of soil and water chemical methods. Inkata Press, Melbourne.

Rayment, G.E., Lyons, D.J., 2011. Soil chemical methods: Australasia. CSIRO Pub., Collingwood, Vic.

Reeder, R.J., Lamble, G.M., Lee, J.F., Staudt, W.J., 1994. Mechanism of SeO42− substitution in calcite: An XAFS study. Geochim. Cosmochim. Acta 58(24), 5639-5646.

Renard, F., Montes-Hernandez, G., Ruiz-Agudo, E., Putnis, C.V., 2013. Selenium incorporation into calcite and its effect on crystal growth: An atomic force microscopy study. Chem. Geol. 340(C), 151-161.

Shock, E.L., Helgeson, H.C., 1988. Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: Correlation algorithms for ionic species and equation of state predictions to 5 kb and 1000°C. Geochim. Cosmochim. Acta 52(8), 2009-2036.

Shrivastava, A., Gupta, V., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron. Young Sci. 2(1), 21-25.

Singh, M., Singh, N., Relan, P.S., 1981. Adsorption and desorption of selenite and selenate selenium on different soils [Normal, calcareous, high organic carbon saline, and alkali]. Soil Sci. 132(2), 134-141.

Smažíková, P., Praus, L., Száková, J., Tremlová, J., Hanč, A., Tlustoš, P., 2017. The effect of organic matter rich amendments on selenium mobility in soil. Pedosphere.

Sparks, D.L., 2003. Environmental soil chemistry. 2nd ed. ed. Academic Press, Amsterdam.

Supriatin, S., Weng, L., Comans, R., 2016. Selenium-rich dissolved organic matter determines selenium uptake in wheat grown on low-selenium arable land soils. Plant and Soil 408(1), 73-94.

Wang, D., Zhou, F., Yang, W., Peng, Q., Man, N., Liang, D., 2017. Selenate redistribution during aging in different Chinese soils and the dominant influential factors. Chemosphere 182, 284-292.

Wang, M., Cui, Z., Xue, M., Peng, Q., Zhou, F., Wang, D., Dinh, Q.T., Liang, D., 2019. Assessing the uptake of selenium from naturally enriched soils by maize (*Zea mays* L.) using diffusive gradients in thin-films technique (DGT) and traditional extractions. Sci. Total Environ. 689, 1-9.

Wright, M.T., Parker, D.R., Amrhein, C., 2003. Critical evaluation of the ability of sequential extraction procedures to quantify discrete forms of selenium in sediments and soils. Environ. Sci. Technol. 37(20), 4709-4716.

Zhang, P., Sparks, D., 1990. Kinetics of selenate and selenite adsorption/desorption at the goethite/water interface. Environ. Sci. Technol. 24(12), 1848-1856.

Zhao, C., Ren, J., Xue, C., Lin, E., 2005. Study on the relationship between soil selenium and plant selenium uptake. Plant and Soil 277(1), 197-206.

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Effect of soil properties on time-dependent fixation (ageing) of selenate

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Figure captions

Fig. 1: The soluble, adsorbed and OM-bound (Se_{Sol}, Se_{Ads} and Se_{Org}, respectively) Se fractions for different soils measured over an ageing period of 300 d. Data show averages Se concentrations and standard errors (n = 4).

Fig. 2: The measured and modelled Se_{Sol} fraction in different soils aged for a period of 300 d. Calcareous and non-calcareous soils are shown on the first and second graphs, respectively.

Fig. 3: The relationship between Se_{Sol} at equilibrium (f_{eq}) and soil pH.

Fig. 4: The chemically-extractable soil (a) soluble and (b) available Se (Se_{Sol} + Se_{Ads}) fractions of aged soils vs. the measured Se concentrations of plants grown in the aged soils. The data points for Inman Valley (InV) and Mallala at the first sampling point (t=1 d) were identified separately in (a) to highlight the overestimation of bioavailability by chemical extraction due to considerable ageing occurring at the beginning at plant growth.

Fig. 5: The relationship between AF_{plant} and AF_{extr}, which are the ageing factors (AF) of Se derived from the ratio of plant Se concentrations and chemically-extracted soil soluble Se at the beginning and end of the ageing period. Ageing factors were log-transformed to homogenise variances.

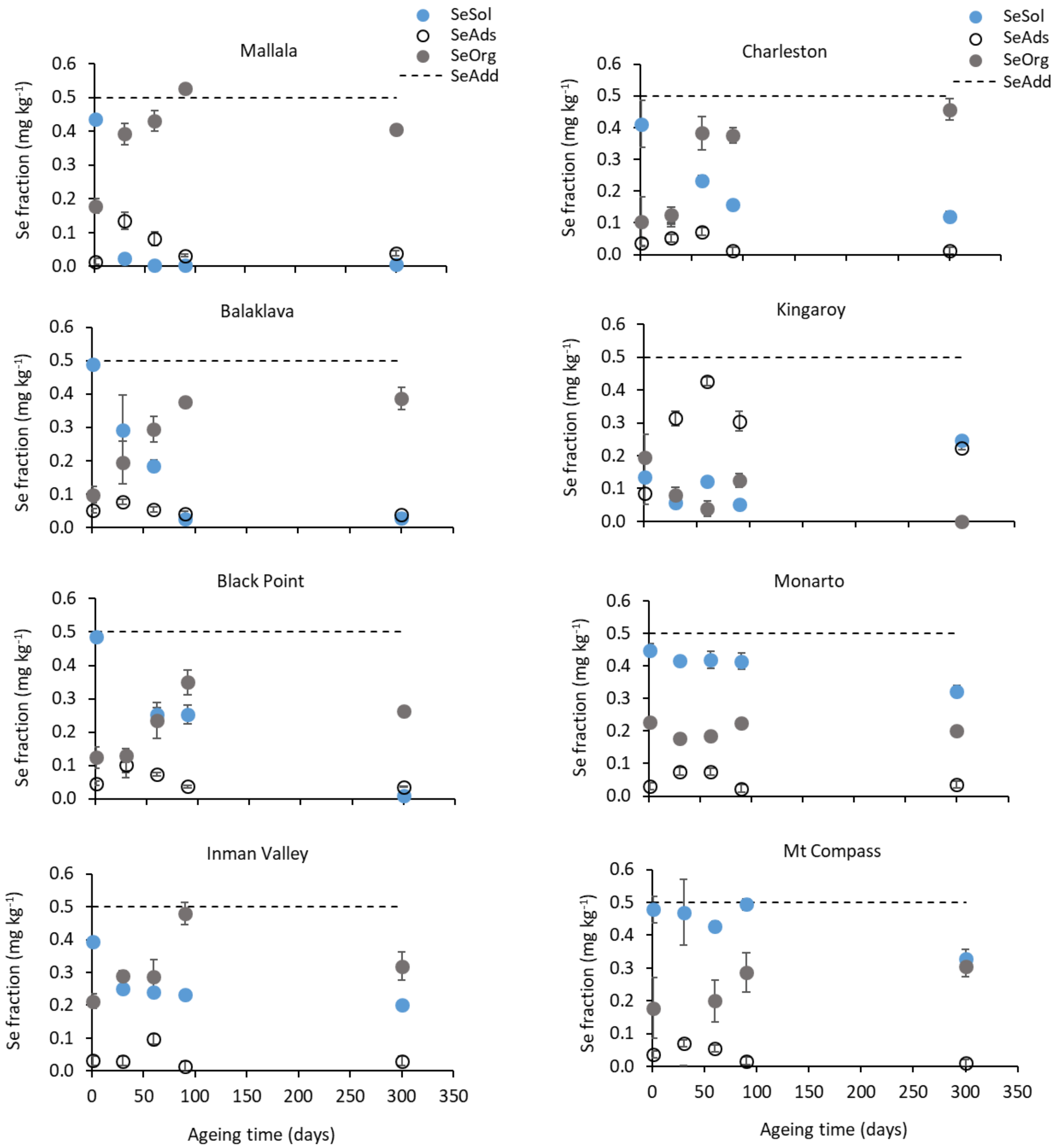
SeSol
SeAds
SeOrg
SeAdd
Mallala
Charleston
Balaklava
Kingaroy
Black Point
Monarto
Inman Valley
Mt Compass
Se fraction (mg kg^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
50
100
150
200
250
300
350
Ageing time (days)

Fig. 1

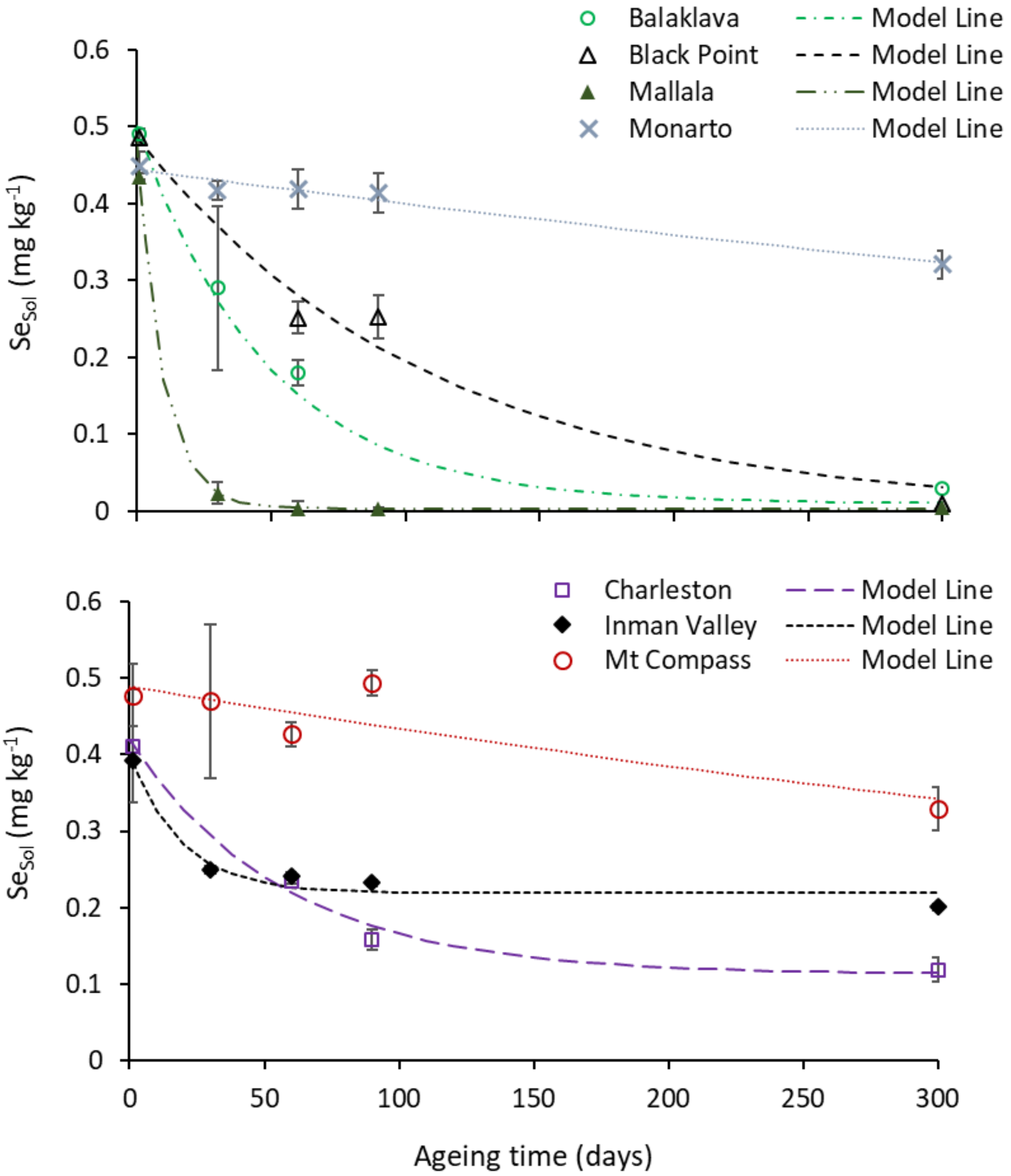
Balaklava
Black Point
Mallala
Monarto
Model Line
Charleston
Inman Valley
Mt Compass
Se_{Sol} (mg kg^{-1})
0
0.1
0.2
0.3
0.4
0.5
0.6
0
50
100
150
200
250
300
Ageing time (days)

Fig. 2

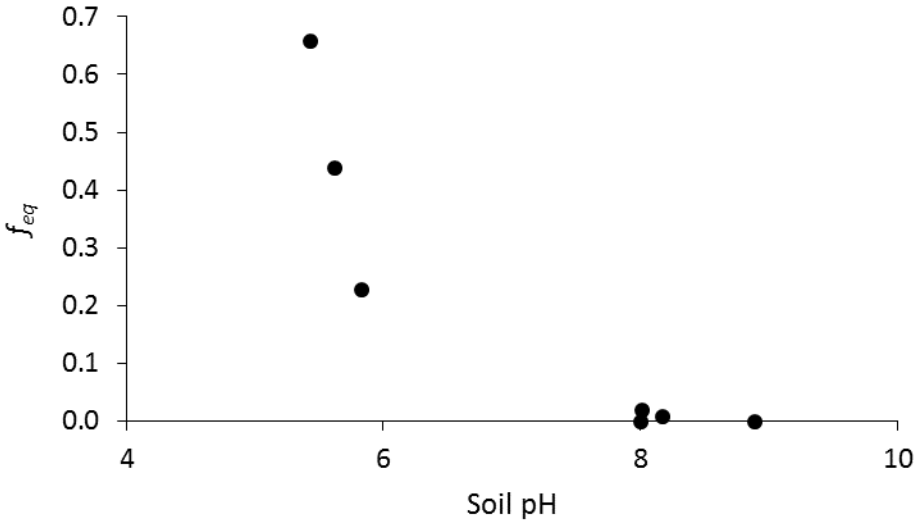

Fig. 3

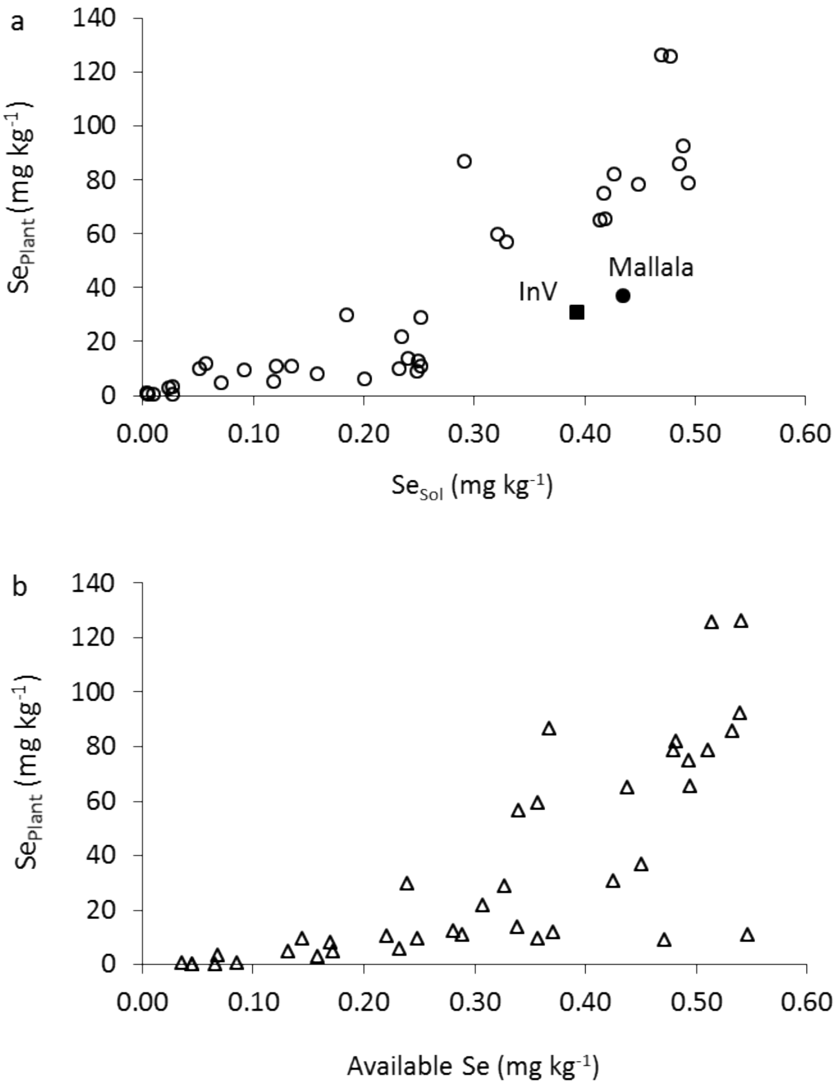
a
Se_Plant (mg kg-1)
140
120
100
80
60
40
20
0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
Se_Sol (mg kg-1)
InV
Mallala
b
Se_Plant (mg kg-1)
140
120
100
80
60
40
20
0
0.00
0.10
0.20
0.30
0.40
0.50
0.60
Available Se (mg kg-1)

Fig. 4

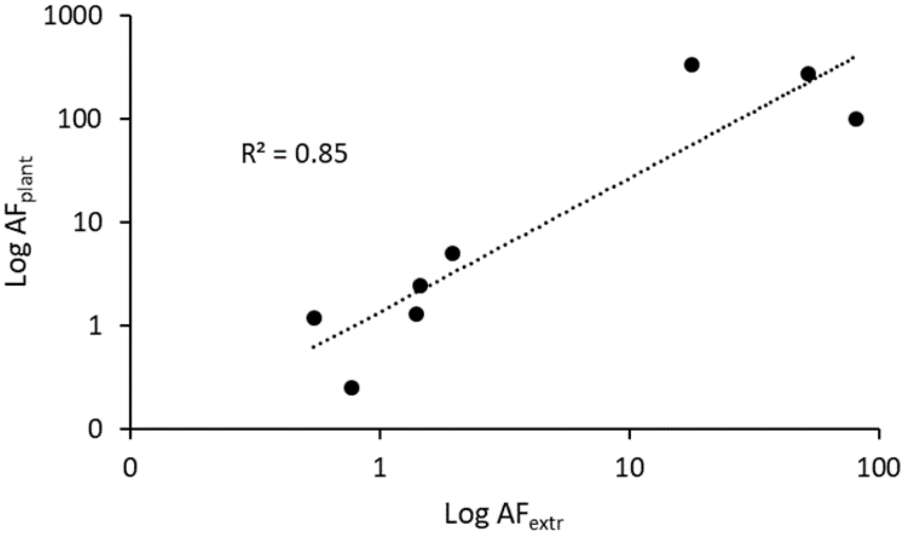

Fig. 5

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: