Agronomic biofortification of cowpea with selenium: effects of selenate and selenite applications on selenium and phytate concentrations in seeds

Running tittle: Effects of selenium in cowpea agronomic biofortification

Vinícius Martins Silva[a], Eduardo Henrique Marcandalli Boleta[b], Juliana Trindade Martins[b], Flavia Lourenço Mendes dos Santos[b], Anne Caroline da Rocha Silva[b], Thomas D. Alcock[c], Lolita Wilson[c], Marco Eustáquio de Sá[b], Scott D. Young[c], Martin R. Broadley[c], Philip J. White[d], and André Rodrigues dos Reis[a,e*]

[a] UNESP - São Paulo State University, Depto. de Produção Vegetal, FCAV, Postal Code 14884-900, Jaboticabal, SP, Brazil

[b] UNESP - São Paulo State University, Postal Code 15385-000, Ilha Solteira, SP, Brazil

[c] School of Biosciences, the University of Nottingham, Sutton Bonington, Leicestershire, LE12 5RD, UK

[d] The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

[e] UNESP - São Paulo State University, Postal Code 17602-496, Tupã, SP, Brazil

*Corresponding author at: UNESP - São Paulo State University, Postal Code 17602-496, Tupã, SP, Brazil

E-mail address: andre.reis@unesp.br (A.R. Reis)

Abstract

Background: Selenium (Se) is a nutrient for animals and humans, and is considered beneficial to higher plants. Selenium concentrations are low in most soils, which can result in a lack of Se in plants, consequently in human diets. Phytic acid (PA) is the main storage form of phosphorus in seeds, able to form insoluble complexes with essential minerals in the monogastric gut. This study aimed to establish optimal levels of Se application to cowpea, with the aim of increasing Se concentrations. The efficiency of agronomic biofortification was evaluated by the application of seven levels of Se (0; 2.5; 5; 10; 20; 40 and 60 g ha^{-1}) from two sources (selenate and selenite) to the soil under field conditions in 2016 and 2017.

Results: Application of Se as selenate led to greater plant Se concentrations than application as selenite in both leaves and grains. Considering a human cowpea consumption of 54.2 g day^{-1}, Se application of 20 g ha^{-1} in 2016 or 10 g ha^{-1} in 2017 as selenate would have provided a suitable daily intake of Se (between 20 and 55 µg day^{-1}) for humans. Phytic acid showed no direct response to Se application.

Conclusion: Selenate provides greater phytoavailability than selenite. The application of 10 g Se ha^{-1} of selenate to cowpea plants could provide sufficient seed Se to increase daily human intakes by 13 to 14 µg d^{-1}.

Keywords: Agronomic biofortification, selenate, selenite, *Vigna unguiculata*, phytate.

1. Introduction

In the years between 1960 and 2007, agricultural food production increased almost three-fold, in order to provide food for a growing population and an increase in consumption per person.1 However, over 800 million people worldwide are still estimated to consume insufficient food to meet dietary energy requirements.2 It is also expected that the global population will increase further from the 2014 estimate of 7.3 billion,1 to 9.7 billion people by 2050.3 In order to nourish this increase in population adequately, whist increasing dietary energy intakes of those currently undernourished, huge increases in global food production will be required over the next few decades.

The prevalence of human malnutrition, defined as inadequate, unbalanced or excessive consumption of macronutrients and/or micronutrients 2 is a significant global issue. Almost half of the world's population is likely to be affected, in particular pregnant women, teenagers and children in developing countries.4 Malnutrition in human populations is linked to recent, intensive plant breeding strategies, which have focused almost entirely on increasing yield. This has resulted in lower concentrations of essential human and animal mineral nutrients in edible crops.5

Humans and animals require at least eighteen mineral elements of which eight are macronutrients, namely nitrogen (N), phosphorus (P), sulphur (S), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), chlorine (Cl), and ten are micronutrients, namely zinc (Zn), iron (Fe), fluorine (F), manganese (Mn), copper (Cu), iodine (I), selenium (Se), molybdenum (Mo), chromium (Cr) and cobalt (Co), among these elements, Fe, Zn, Cu, Ca, Mg, Se and I are often lacking in human diets.6

As a human and animal nutrient, Se acts as an antioxidant and is involved in the immune system and thyroid function.7 In plants Se can promote growth, and act in oxidative stress combat, being considered a benefit trace element.8, 9, 10 Is estimated that more than one billion people may suffer from Se deficiency.11 Selenium deficiency presents the third highest deficiency risk of any mineral micronutrient in Africa.12

The Se availability in the soil, determine its concentration in edible parts of agricultural products.13 Increasing concentrations of soil Se through the application of Se-fertilizers increases Se uptake by plants and improves nutritional quality of food.8

The addition of Se together with NPK fertilizers in agricultural areas seems to be an effective and safe way to reduce Se deficiency in human and animal nutrition, as evident from decades of monitoring programs carried out in Finland.14, 15 Different sources of Se such as selenate and selenite present distinct behaviours in soil and plants. For instance, the uptake, assimilation and translocation of selenate is faster than that of selenite.16 Selenate is the main Se form in oxygenated soils, and selenite is the main Se source in anaerobic environments, such as paddy soils.8 Hence, the Se source used must be considered carefully in agronomic biofortification strategies. Care must also be taken when aiming to increase Se concentrations of agricultural products, as although Se is essential to humans, there is a narrow range between deficient and toxic levels.11 For instance, a minimum Se intake of 20 µg day^{-1} is necessary to prevent Keshan disease,17, 18 the Se intake recommended for adults is 55 µg day^{-1}19 and the tolerable upper intake level for adults is 400 µg day^{-1}.7

Phytic acid (PA) (or myo-inositol hexaphosphate, InsP6 or IP6) is the main compound of phosphorus (P) storage in seeds, comprising between 60 and 90% of all P in cereals, oilseeds and nuts.20, 21 The bioavailability Fe, Zn, Cu and Mg in human diets is often reduced by the presence of PA, which is considered an antinutrient.20, 6 For this reason, there are commonly severe mineral micronutrient deficiencies in populations with diets composed primarily of cereals, particularly in vulnerable groups such as children and pregnant women from poor regions that commonly ingest low amounts of bioavailable Ca, Fe, Mg and Zn.22 Thus, strategies to reduce PA concentrations in edible part of plants are also needed in order to enhance the nutritional quality of foods.

Selenate is taken up from soil by high-affinity, H^+/sulphate symporters in the plasma membrane of root cells and selenite is taken up either as $HSeO_3$ by members of the phosphate transporter Pht1 family or as H_2SeO_3 through aquaporins.23 Thus, increasing Se in the rhizosphere solution could compete with sulphate and phosphate for uptake. A reduction in P uptake can influence the synthesis of PA and, consequently, the concentrations of PA and cations associated with it in the seed.

Leguminous plants provide on average 2 µg of Se capita^{-1} day^{-1}. 12 Cowpea (*Vigna unguiculata* (L.) Walp.) is a fabaceae with an important role as source of minerals and proteins, complementing other foods, such root vegetables, cereals and tuberous in diets from Latin American, Asian and African developing countries.24 Due to cowpea's nutritional qualities, adaptability to arid regions and tolerance to drought, it is considered a suitable crop for cultivation in hot, arid regions.25

The biofortification of edible crops with Se may affect the concentrations of other essential mineral elements, including macronutrients and micronutrients, and antinutrients, such as PA, in seed. In addition, Se biofortification using different Se-fertilisers, such as selenate or selenite, could have contrasting effects on seed nutritional quality. In this paper the effects of Se biofortification using selenate or selenite on the elemental composition and PA concentrations of cowpea seeds were studied. A better understanding of factors that influence Se concentration in cowpea seeds will inform strategies for increasing the dietary intake of Se safely using this legume.

2. Materials and methods

2.1. Experimental conditions

The experiment was realized at the São Paulo State University (UNESP) Farm, Mato Grosso do Sul State, Brazil. Daily rainfall and mean maximum and minimum temperatures recorded during the experiment can be found in appendix A. The experimental area soil was classified as Rhodic Haplusfox according to the Soil Survey

Staff26 with the following chemical characteristics determined according to Raij et al.27: pH: 5.5; P, S, B, Cu, Fe, Mn and Zn: 34, 8, 0.19, 2.7, 19, 12.4 and 6.1 mg dm^{-3} respectively; K; Ca; Mg; H+Al and cation exchange capacity: 2.7, 14, 14, 26 and 56.7 mmol$_c$ dm^{-3} respectively; V%: 54 and organic matter: 18 g dm^{-3}. Readily available soil Se concentration was 3.6 µg dm^{-3}. To determine Se concentration, 4 g of air dried soil was added to a 50 mL centrifuge tube along with 20 mL of 0.01M KNO_3. The solution was shaken in a rotary shaker for 2 hours, then centrifuged for 30 min at 3500 rpm. After centrifugation, 9 mL of the supernatant was pippeted into a < 0.22 µm syringe filter. The supernatant was filtred into a tube containing 10% tetramethylammonium hydroxide (TMAH) and 0.1 M KH_2PO_4 for determination of readily available Se using inductively coupled plasma-mass spectrometry (ICP-MS; Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany).

The adsorption pattern for selenate and selenite in the soil of the present study was determined (figure 1). To determine the adsorption, 1 g soil samples were weighed, and 20 mL of 0.01 M $Ca(NO_3)_2$ containing different concentrations of Se: 0 (control), 2.5; 5.0; 7.5 and 12.5 µg L^{-1} as sodium selenate or sodium selenite were added to each sample. Soil samples with solutions were stored in 50 mL polyethylene falcon tubes and keep shaking in a rotating shaker at 20 rpm for 48 hours. After shaking, the samples were centrifuged for 15 minutes at 3000 rpm. A 9.6 mL aliquot was collected from each of the samples and filtered through a 0.22 µm filter. To this, 0.4 mL of 50 % HNO_3 was added. The concentration of Se in each sample was obtained using ICP-MS.

2.2. Growth of plant material and treatment application

Experiments were performed in two agricultural years (2016 and 2017). Seeds were sown on October 18th, 2016 (first year) and March 23rd, 2017 (second year), the sowing density of 11.2 seeds m^{-1} was stablished. Fertilization consisted of 33 kg ha^{-1}

KCl (20 kg ha^{-1} K_2O) and 110 kg ha^{-1} single superphosphate (20 kg ha^{-1} P_2O_5) applied in the planting furrow. The experimental design was a complete randomized block in a factorial scheme (7 x 2), in which four replicates of seven Se rates (0; 2.5; 5.0; 10; 20; 40 and 60 g ha^{-1}) from two sources (sodium selenate and sodium selenite) were applied. The experimental plot consisted of 5 m rows with an interrow spacing of 0.45 m between them.

The cowpea variety used was BRS Tumucumaque, for inoculation, a peat inoculum specific for cowpea was used (2.0x10^{9} colony forming units g^{-1}, strain SEMIA 6462), at 8 g kg^{-1} of seed. Prior to inoculation the seeds were treated, at the rate of 2 mL product per kg of seeds, with the following commercial products: thiophanate-methyl (225 g L^{-1}), fipronil (250 g L^{-1}) and pyraclostrobin (25 g L^{-1}). Emergence began on October 22nd, 2016, four days after sowing (4 DAS), and on March, 28th, 2017, five days after sowing (5 DAS).

Applications of Se were made 33 days after emergence of seedlings (DAE) in 2016, and 35 DAE in 2017. All the Se required for each treatment across all four replicates was weighed and diluted in 2 L of water, generating a stock solution for each treatment. The stock solution was then subdivided into four portions of 500 mL each. Solutions were applied to the soil. In each plot, 100 mL of solution was applied to each line.

Pest control was as follows: In 2016, pest control measures were undertaken using abamectin (0.5 L ha^{-1}) at 12 and 23 DAE, bentazone (0.8 L ha^{-1}) and imidacloprid (0.4 L ha^{-1}) at 23 DAE, and haloxyfop-p-methyl (0.3 L ha^{-1}) at 33 DAE. In 2017, pest control measures were undertaken 10 DAE (clethodim + alquilbenzene 0.4 L ha^{-1}, beta-cyfluthrin 0.15 L ha^{-1}, abamectin, 0.5 L ha^{-1}), 14 DAE (benthazon 1.2 L ha^{-}

^{1}), 15 DAE (deltamethrin 0.06 L ha^{-1}, beta-cyfluthrin+imidacloprid 0.87 L ha^{-1}) and 28 DAE (beta-cyfluthrin 0.15 L ha^{-1}, abamectin, 0.50 L ha^{-1}).

2.3.Sampling.

For mineral analysis, the third trifoliate leaves were collected, at 23 days after Se supply in 2016 (59 DAE) and 24 days after Se supply in 2017 (56 DAE). The plant material was removed, dried in an oven at 40 °C for 72 hours until a constant dry mass was achieved, and ground in a Wiley mill (Marconi, MA 340, Piracicaba, Brazil) using 1 mm sieve.

Seed was harvested 77 DAE in 2016 and 72 DAE in 2017. All pods were harvested manually, for the process two homogeneous rows from each plot was selected. The following agronomic parameters were analysed: yield, shoot dry mass (SDM), pod length (PL), number of pods per plant (NPP) and number of seeds per pod (NSP).

2.4. Digestion and mineral analysis of leaf and seed samples

Subsamples of 0.20 g dry weight (DW) of leaf or seed material was digested and analysed by ICP-MS according to Thomas et al.28 Sample processing was undertaken using Qtegra™ software (Thermo Fisher Scientific).

2.5. Extraction and phytic acid analysis of seeds.

To determine phytic acid (PA) concentrations of seed samples, approximately 1.0 g DW seed subsamples were weighed and placed into 50 mL conical tubes (SARSTEDT). To each tube, 20 mL of 0.66 M HCl was added, and tubes were put in a rotary shaker to shake overnight at 25 rpm. After this, 1 mL of the homogenized solution was transferred to 2 mL safe lock microtubes (Eppendorf, Hamburg, Germany) and centrifuged at 13,000 rpm for 10 minutes. After centrifugation, 0.5 mL of the supernatant was transferred to a new 2 mL safe lock microtubes and neutralized with

0.5 mL of 0.75 M NaOH. This extract was used to perform an enzymatic dephosphorylation reaction, in order to estimate PA concentration from the difference between total phosphorus and free phosphorus assayed using the Phytic Acid (Total Phosphorus) Assay Kit (Megazyme) following the manufacturer's instructions.

The absorbance of phosphomolybdate was read in 96 well plate readers (Thermo Scientific, Waltham, MA, USA) at 650 nm in an EL808 absorbance reader (BIOTEK). One operational blank (ultrapure water), one oat flour control and one phosphorus calibration curve (0, 0.5, 2.5, 5 and 7.5 µg mL-1), were included in each plate.

2.6. Statistical analysis

The datased was submitted to Anderson-Darling normality tests. Leven's test was used to evaluate homogeneity. After variance analysis (F test), a Tukey test at 5 % was used to compare differences between treatments, or a regression, depending on the variable analysed. Agroestat and Minitab softwares were used to perform the analysis, and the software SigmaPlot 12.5 was used to prepare the graphs presented.

3. Results

There was no influence of sodium selenate or sodium selenite application on cowpea yield either in the first or second year of field trials (figure 2a-b). In the first year the average yield was 1770 kg ha^{-1} and in the second year the average yield was 1220 kg ha^{-1}. The application of neither sodium selenite nor sodium selenate had any effect on shoot dry weight in either year of cultivation (figure 2c-d). Similarly, pod length (PL), number of pods per plant (NPP) and number of seeds per pod (NSP) were unaffected by application of sodium selenate or sodium selenite (appendix B).

An interaction was observed between the sources and concentrations of Se applications on leaf Se concentration in both the first and second years (figure 3). In the first year, leaf Se concentration increased with increasing selenate and selenite applications. In the first year, the application of selenate resulted in greater leaf Se concentration than the application of selenite at equivalent rates of 5, 10 and 40 g Se ha^{-1} (figure 3a) and leaf Se concentration ranged from 0.06 to 0.52 mg kg^{-1}. Similar effects were observed in the second year when leaf Se concentration again increased with increasing selenate or selenite applications. The application of selenate resulted in greater leaf Se concentrations than the application of selenite at equivalent rates of 10, 20, 40 and 60 g Se ha^{-1} (figure 3a) and Se concentration ranged from 0.2 to 2.66 mg kg^{-1}. Selenium application at rates of 2.5, 10, 40 and 60 g ha^{-1} reduced S leaf concentrations in the second year, regardless of the source (appendix C).

Seed Se concentration was affected by the source and concentration of Se applied in both the first and second years (figure 4). In the first year, seed Se concentration increased in response to increasing selenate and selenite application, and seed Se concentration ranged from 0.05 to 0.83 mg kg$^{-1.}$ The application of selenate resulted in greater seed Se concentration than the application of selenite at equivalent rates of 10, 20, 40 and 60 g Se ha^{-1} (figure 4a). In the second year, seed Se concentration increased with increasing selenate application, and seed Se concentration ranged from 0.04 to 2 mg kg^{-1}. The application of selenate resulted in greater seed Se concentrations than the application of selenite at equivalent rates of 20, 40 and 60 g Se ha^{-1} (figure 4b).

Seed PA concentration was affected by both the source and concentration of Se applied in both the first and second experiments years (figure 5). In the first year, the application of selenate led to a greater PA concentration than the application of selenite at an equivalent rate of 5, 40 and 60 g Se ha^{-1}, and the application of selenite led to a higher seed PA concentration than the application of selenate at an equivalent rate of 2.5 and 10 g Se ha^{-1} (figure 5a). In the second year, the application of selenite resulted in a greater seed PA concentration than the application of selenate at equivalent rates of 2.5 and 40 g Se ha^{-1} (figure 5b).

4. Discussion

The application of Se resulted in a substantial increase in Se concentrations in leaves and seed of cowpea plants (figures 3 and 4). However, yield, shoot dry mass, pod length, number of pods per plant and numbers of seeds per pod were not affected by Se application (figures 2). A beneficial effect of Se on biomass, yield and other agronomic parameters is occasionally observed in plant subject to abiotic stress. For example, the application of Se has been shown to increase growth and productivity in salt stressed cowpea plants.29 In other species, such as mungbean (*Vigna radiata* L. Wilczek), the application of Se to salt stressed plants has been shown to improve reproductive function30 and the application of Se to wheat plants exposed to ultraviolet-B radiation resulted in increased spike length, weight per spike and yield.31 The beneficial effect of Se to stressed plants might be a consequence of its role in mitigating oxidative stress.8 In the present study, as plants were not exposed to stress, neither sodium selenate nor sodium selenite influenced the yield or biomass of cowpea.

In the present study, the application of Se led to greater Se concentrations in leaves and seed of cowpea than the application of selenite at the same rate (figures 3 and 4). This is consistent with many previous studies reporting that the application of selenate leads to greater tissue Se concentrations than the application of selenite at an equivalent rate, for example in shoots of Indian mustard, and in roots, stems, leaves and grain of wheat 32, 33 and in seeds of lentils.34

In plants, the uptake of selenate and its translocation from roots to shoots is faster than that of selenite.16 Indeed, only a minor fraction of the selenite taken up by roots is translocated from roots to shoots since it is readily converted into organic forms.8 The conversion of selenate into organic Se forms is slower, and this allows greater mobility in plants.18 Thus, the greater Se concentrations in leaves and seeds of cowpea plants receiving selenate rather than an equal application of selenite is likely to be explained, in part, by the greater translocation efficiency of selenate than selenite. The behavior of selenate and selenite in the soil might also contribute to the differences in tissue Se concentrations when equivalent amounts of selenate and selenite were applied. Selenite was more strongly adsorbed by the soil than selenate (figure 1) and is, therefore, less phytoavailable.

Wide variation in seed Se concentration was observed among the Se treatments and the two cultivation years, ranging from approximately 0.5 to 2 mg kg^{-1} (Figure 4). The recommended dietary allowance for adult humans is 55 µg day^{-1}19 and the tolerable upper intake level is 400 µg day^{-1}.7 There is no daily intake recommendation set for cowpea, although some recommendations for legume intake have been made, ranging from 50 to 81 g day^{-1}.35

Brazilian eating habits vary widely due to social, economic and geographical differences, but foods such rice, cow milk and eggs are commonly consumed throughout the country. The mean Se concentrations in Brazilian rice, cow milk and eggs are 0.31, 0.08 and 0.21 µg g^{-1}, respectively.36 The daily intake of rice, cow milk and eggs in the northeastern region of Brazil are 142, 33 and 16 g, respectively.37 Multiplying Se concentration by the daily consumption of each of these foods, the total Se daily intake from them is about 25 µg day^{-1} per capita. Subtracting this value from the recommended dietary allowance of 55 µg Se d^{-1}, it is possible to establish a guide for the daily Se intake provided by cowpea seeds of 30 µg d^{-1}.

The consumption of cowpea beans varies widely. In African countries, consumption varies from 1.5 kg per capita per year in Senegal to 18 kg per capita per year in Nigeria.38 In the northeastern region of Brazil, Se consumption per capita per year is 19.79 k,39 which corresponds to 54.22 g of cowpea beans per day. The daily intake of Se from cowpea can be calculated by multiplying the daily cowpea consumption by the Se concentration in each treatment (figure 6).

Application rates of 40 and 60 g Se ha^{-1} of sodium selenate in the first year, and 20, 40 and 60 g Se ha^{-1} of sodium selenate in the second year would have provided a greater Se intake than the guide value of 30 µg Se d^{-1} from consuming 54.20 g d^{-1} cowpea seed.

Although phosphate and selenite can compete for uptake by plant roots,11 the application of neither selenate nor selenite influenced P concentration in seeds (appendix F). Similarly, there was no obvious relationship between the PA concentration in seeds and the application of Se as either selenate or selenite (Figure 5). Furthermore, the concentrations in seeds of most elements to which PA might bind were unaffected by the application of selenate or selenite (appendix D and E). Thus, Se biofortification of cowpea seeds would not appear to impact negatively on the bioavailability of other human and animal nutrients.

5. Conclusions

Selenium uptake and accumulation in leaves and seeds of cowpea plants depends upon the Se source (selenate or selenite). Less selenate was absorbed to Brazilian soils than selenite, providing greater phytoavailability for uptake by roots. The application of 10 g Se ha^{-1} of sodium selenate to cowpea plants could provide sufficient seed Se to increase daily human Se intakes by 13 to 14 µg d^{-1}, without affecting plant biomass or crop yield. Furthermore, the application of 10 g Se ha^{-1} of sodium selenate to cowpea plants had no effect on the concentrations of most mineral elements essential to human nutrition in seeds and did not affect the concentrations of PA in seed. Thus, the present study can inform agronomic strategies to biofortify cowpea seeds with Se for human nutrition.

6. Acknowledgments

We acknowledge FAPESP (São Paulo Research Foundation) for supporting this research with financial resources provided by process numbers: 16/19773-8 and 17/21950-8. ARR also thanks the National Council for Scientific and Technological Development (CNPq) for the research fellowship (grant 309380/2017-0). PJW was supported by the Rural and Environment Science and Analytical Services Division of the Scottish Government.

7. Conflict of interest

We have no conflicts of interest to disclose.

8. References

1 Cazalis, V.; Loreau, M.; Henderson, K. (2018). Do we have to choose between feeding the human population and conserving nature? Modelling the global dependence of people on ecosystem services. Sci Total Environ, 634:1463-1474. doi: 10.1016/j.scitotenv.2018.03.360

2 FAO, IFAD, UNICEF, WFP and WHO. (2017). The State of Food Security and Nutrition in the World 2017. Building resilience for peace and food security. FAO, Rome.

3 United Nations, 2015. World Population Prospects: Key Findings and Advance Tables. Department of Economic and Social Affairs, Population Division, New-York.

4 Das, J. K.; Lassi, Z. S.; Hoodbhoy, Z.; Salam R, A. (2018). Nutrition for the next generation: older children and adolescents. Ann Nutr Metab, 72(suppl 3): 56-64. doi: 10.1159/000487385

5 White, P. J.; Bradshaw, J. E.; Dale, M. F. B.; Ramsay, G.; Hammond, J. P.; Broadley, M. R. (2009). Relationships between yield and mineral concentrations in potato tubers. HortScience, 44:6-11.

6 White, P. J. (2016a). Biofortification of Edible Crops. ELS, eLS, pp.1-8. doi:10.1002/9780470015902.a0023743.

7 Fairweather-Tait, S. J.; Bao, Y.; Broadley, M. R.; Collings, R.; Ford, D.; Hesketh, J. E.; Hurst, R. (2011). Selenium in human health and disease. Antioxid Redox Sign, 14: 1337–1383. doi: 10.1089/ars.2010.3275

8 White, P. J. (2016b). Selenium accumulation by plants. Ann Bot-London, 117:217–235. doi: 10.1093/aob/mcv180

9 Reis, A. R.; Barcelos, J. P. Q.; Osório, C. R. W. S.; Santos, E. F.; Lisboa, L. A. M.,; Santini, J. M. K.; Santos, M. J. D.; Furlani Junior, E.; Campos, M.; Figueiredo, P. A. M.; Lavres, J.; Gratão, P. L. (2017). A glimpse into the physiological: biochemical and nutritional status of soybean plants under Ni-stress conditions. Environ Exp Bot, 144:78–87. doi: 10.1016/j.envexpbot.2017.10.006

10 Silva, V. M.; Boleta, E. H. M.; Lanza, M. G. D. B.; Lavres, J.; Martins, J. T.; Santos, E. F.; Dos Santos, F. L. M.; Putti, F. F.; Junior, E. F.; White, P. J.; Broadley, M. R.; Carvalho, H. W. P. D.; Reis, A. R. D. (2018). Physiological, biochemical, and ultrastructural characterization of selenium toxicity in cowpea plants. Environ Exp Bot, 150:172–182. doi: 10.1016/j.envexpbot.2018.03.020

11 Schiavon, M.; Pilon-Smits, E. A. H. (2017). The fascinating facets of plant selenium accumulation– biochemistry, physiology, evolution and ecology. New Phytol. 213: 1582–1596; doi: 10.1111/nph.14378

12 Joy, E. J. M.; Ander, E. L.; Young, S. D.; Black, C. R.; Watts, M. J.; Chilimba, A. D. C.; Chilima, B.; Siyame, E. W. P.; Kalimbira, A. A.; Hurst, R.; Fairweather-Tait, S. J.; Stein, A. J.; Gibson R. S.; White, P. J.; Broadley, M. R. (2014). Dietary mineral supplies in Africa. Physiol Plantarum, 151:208–229. doi: 10.1111/ppl.12144

13 Joy, E. J. M.; Broadley, M. R.; Young, S. D.; Black, C. R.; Chilimba, A. D. C.; Ander, E. L.; Watts, M. J. (2015). Soil type influences crop mineral composition in Malawi. Sci Total Environ, 505:587–595. doi: 10.1016/j.scitotenv.2014.10.038

14 Hartikainen, H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. J Trace Elem Med Bio , 18:309-318. doi: 10.1016/j.jtemb.2005.02.009

15 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 D, 19:209-228. doi: 10.1080/08910600701698986

16 Gupta M.; Gupta, S. (2017). An Overview of Selenium Uptake, Metabolism, and Toxicity in Plants. Front Plant Sci, 7:2074. doi: 10.3389/fpls.2016.02074

17 Roman, M.; Jitaru, P.; Barbante, C. (2014). Selenium biochemistry and its role for human health. Metallomics 6, 25-54. doi: 10.1016/S0140-6736(11)61452-9

18 Natasha; Shahid, M.; Niazi, N. K.; Khalid, S.; Murtaza, B.; Bibi, I.; Rashid, M. I. (2018). A critical review of selenium biogeochemical behavior in soil-plant system with an inference to human health. Environ Pollut, 234:915-934. doi: 10.1016/j.envpol.2017.12.019

19 Institute Of Medicine. (2000). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. The National Academies Press, Washington, DC. doi: 10.17226/9810

20 Raboy, V. (2003). *myo*-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochemistry, 64:1033–1043. doi: 10.1016/S0031-9422(03)00446-1

21 Bohn, L.; Meyer, A. S.; Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. Journal of Zhejiang University SCIENCE B, 9:165-191. doi:10.1631/jzus.B0710640

22 Weaver, C. M.; Kannan, S. (2002). Phytate and mineral bioavailability. In: Reddy N R., Sathe S K. (eds) Food phytates. CRC Press, Boca Raton, 211-223. doi: 10.1201/9781420014419.ch13

23 White, P. J. (2018). Selenium metabolism in plants. Biochim Biophys Acta - General Subjects, 1862:2333-2342. doi:10.1016/j.bbagen.2018.05.006.

24 Manzeke, M. G.; Mtambanengwe, F.; Nezomba, H.; Watts, M. J.; Broadley, M. R.; Mapfumo, P. (2017). Zinc fertilization increases productivity and grain nutritional quality of cowpea (*Vigna unguiculata* [L.] Walp.) under integrated soil fertility management. Field Crop Res, 213:231–244. doi: 10.1016/j.fcr.2017.08.010

25 Hall, A. E. (2012). Phenotyping cowpeas for adaptation to drought. Front Physiol, 3:155. doi: 10.3389/fphys.2012.00155

26 Soil Survey Staff. (2014). Keys to Soil Taxonomy, twelfh ed. USDA. Natural Resources Conservation Service, Washington, DC

27 Raij, B. V.; De Andrade, J. C.; Cantarella, H.; Quaggio, J. A. (1997). Análise Química Para Avaliação da Fertilidade de Solos Tropicais. Instituto Agronômico de Campinas, Campinas.

28 Thomas, C. L.; Alcock, T. D.; Graham, N. S.; Hayden, R.; Matterson, S.; Wilson, L.; Young, S. D.; Dupuy, L. X.; White, P. J.; Hammond, J. P.; Danku, J. M. C.; Salt, D. E.; Sweeney, A.; Bancroft, I.; Broadley, M. R. (2016). Root morphology and seed and leaf ionomic traits in a *Brassica napus* L. diversity panel show wide phenotypic variation and are characteristic of crop habit. Bmc Plant Biol, 16:214. doi: 10.1186/s12870-016-0902-5

29 Manaf, H. H. (2016). Beneficial effects of exogenous selenium, glycine betaine and seaweed extract on salt stressed cowpea plant. Ann Agr Sci, 61:41–48. doi: 10.1016/j.aoas.2016.04.003

30 Kaur, S.; Nayyar, H. (2015). Selenium fertilization to salt-stressed mungbean (Vigna radiate L. Wilczek) plants reduces sodium uptake, improves reproductive function, pod set and seed yield. Sci Hortic-Amsterdam. 197:304–317. doi: 10.1016/j.scienta.2015.09.048

31 Yao, X.; Jianzhou, C.; Xueli, H.; Binbin, L.; Jingmin, L.; Zhaowei, Y. (2013). Effects of selenium on agronomical characters of winter wheat exposed to enhanced ultraviolet-B. Ecotox Environ Safe. 92:320–326. doi: 10.1016/j.ecoenv.2013.03.024

32 Eiche, E.; Bardelli, F.; Nothstein, A. K.; Charlet, L.; Göttlicher, J.; Steininger, R.; Dhillion, K. S.; Sadana, U. S. (2015). Selenium distribution and speciation in plant parts of wheat (*Triticum aestivum*) and Indian mustard (*Brassica juncea*) from a seleniferous area of Punjab, India. Sci Total Environ, 505 :952–961. doi: 10.1016/j.scitotenv.2014.10.080

33 Ali, F.; Peng, Q.; Wang, D.; Cui, Z.; Huang, J.; Fu, D.; Liang, D. (2017). Effects of selenite and selenate application on distribution and transformation of selenium fractions in soil and its bioavailability for wheat (*Triticum aestivum* L.). Environ Sci Pollut R, 24:8315-8325. doi: 10.1007/s11356-017-8512-9

34 Ekanayake, L. J.; Thavarajah, D.; Vial, E.; Schatz, B. Mcgee, R.; Thavarajah, P. (2015). Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. Field Crop Res, 177: 9-14. doi: 10.1016/j.fcr.2015.03.002

35 Venter, C. S.; Vorster, H. H.; Ochse, R.; Swart, R. (2013). “Eat dry beans, split peas, lentils and soya regularly”: a food-based dietary guideline. South Afr J Clin Nutr, 3(suppl):S36-S45.

36 Santos, M.; Silva Jr, F. M R.; Muccillo-Baisch, A. L. (2017). Selenium content of Brazilian foods: A review of the literature values. J Food Compos Anal, 58:10–15. doi: 10.1016/j.jfca.2017.01.001

37 Instituto Brasileiro De Geografia E Estatistica. (2009). Prevalência de consumo alimentar e consumo alimentar médio per capita, por Grandes Regiões, segundo os alimentos - Brasil - período 2008-2009. Available on:

https://ww2.ibge.gov.br/home/estatistica/populacao/condicaodevida/pof/2008_2009_analise_consumo/defaulttab_zip_alimentos.shtm.

38 Gómez, C.; Mejía, D. (2004). COWPEA: Post-Harvest Operations. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. 71p.

39 Freire Filho, F. R.; Ribeiro, V. Q.; Rodrigues, J. E. L. F.; Vieira, P. F. M. J. (2017). A Cultura: aspectos socieconômicos. In: Do Vale, J C., Bertini, C., Borém A. (eds.). Feijão-caupi: do plantio à colheita. Editora UFV, Viçosa, 9-35.

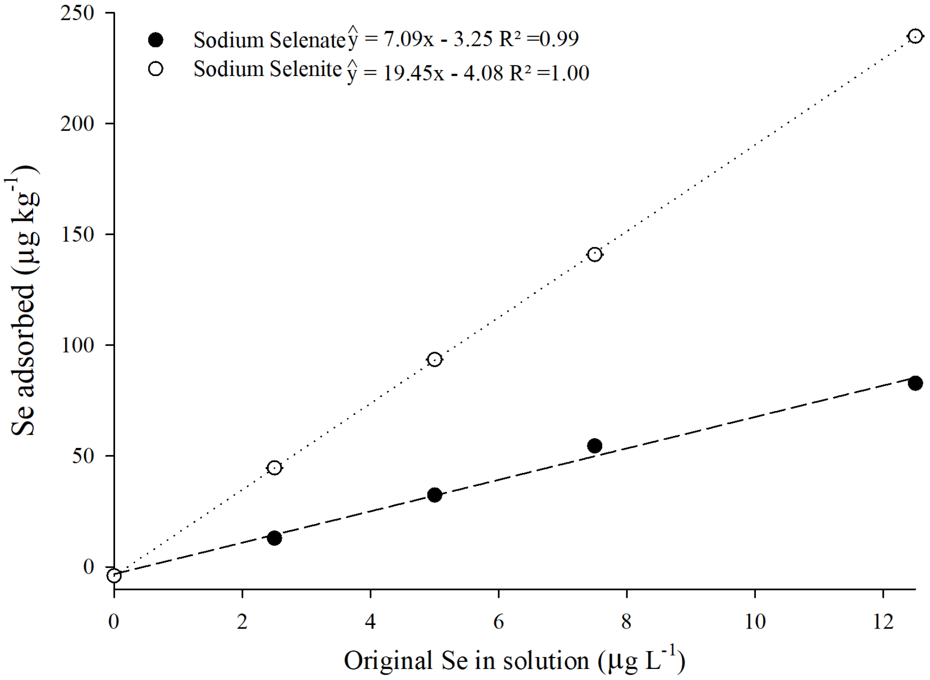


Figure 1. Sodium selenate and sodium selenite adsorption in soils from Selviria city, State of Mato Grosso do Sul, Brazil.

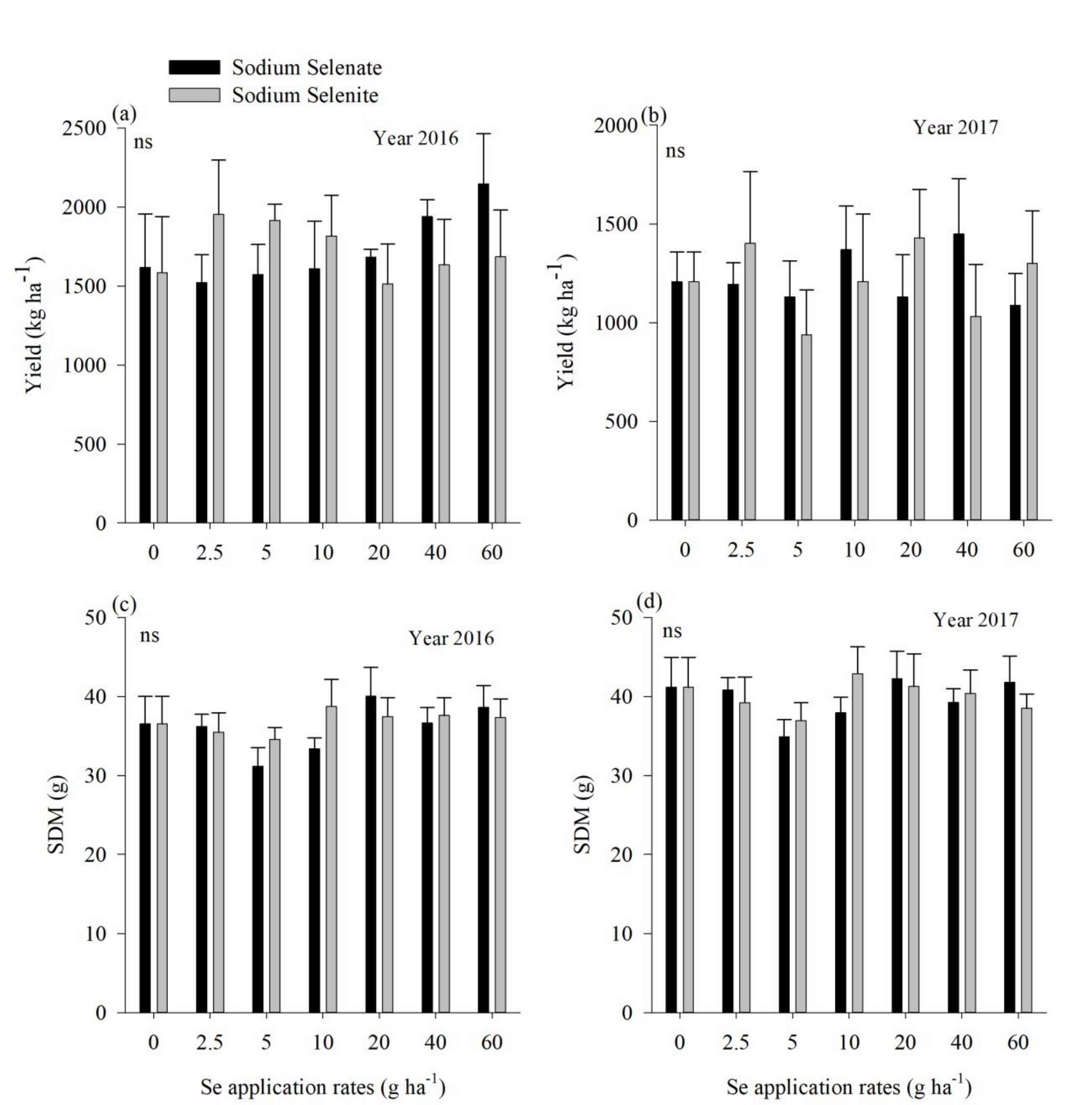


Figure 2. The effect of applying sodium selenate or sodium selenite on the yield of cowpea plants in the first (a) and second (b) year of trials and shoot dry weight in first (c) and second (d) year of trials. The standard error of the mean (n = 4) is indicated by error bars. ns = not significant. CV (%) = 23.82 (a); 34.06 (b); 15.13 (c) and 15.10 (d).

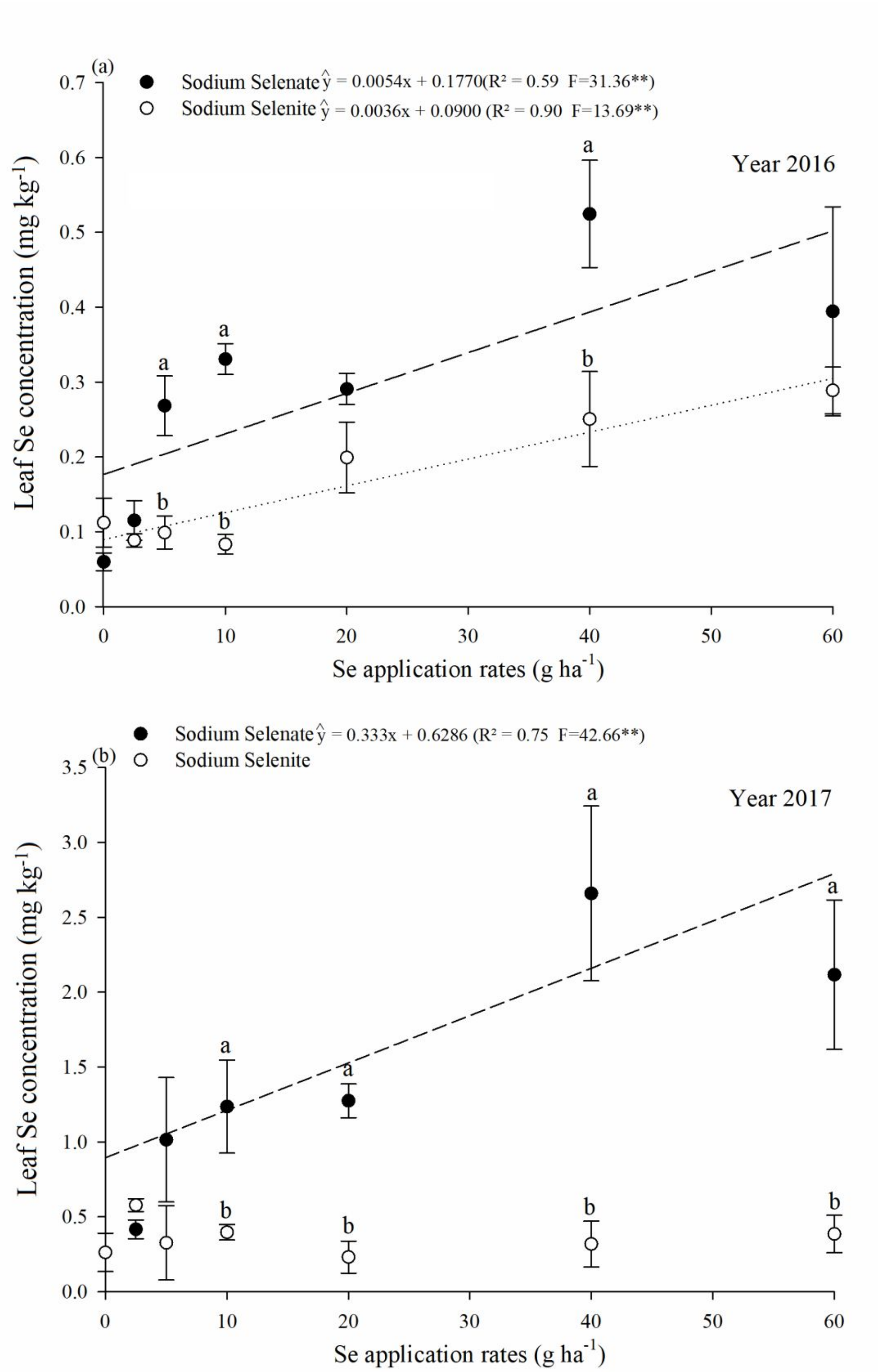


Figure 3. The effect of applying sodium selenate or sodium selenite on leaf Se concentration in the first (a) and second (b) year. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate differences between the means according to a Tukey test ($p \leq 0.05$). CV (%) = 48.03 (a); 68.47 (b).

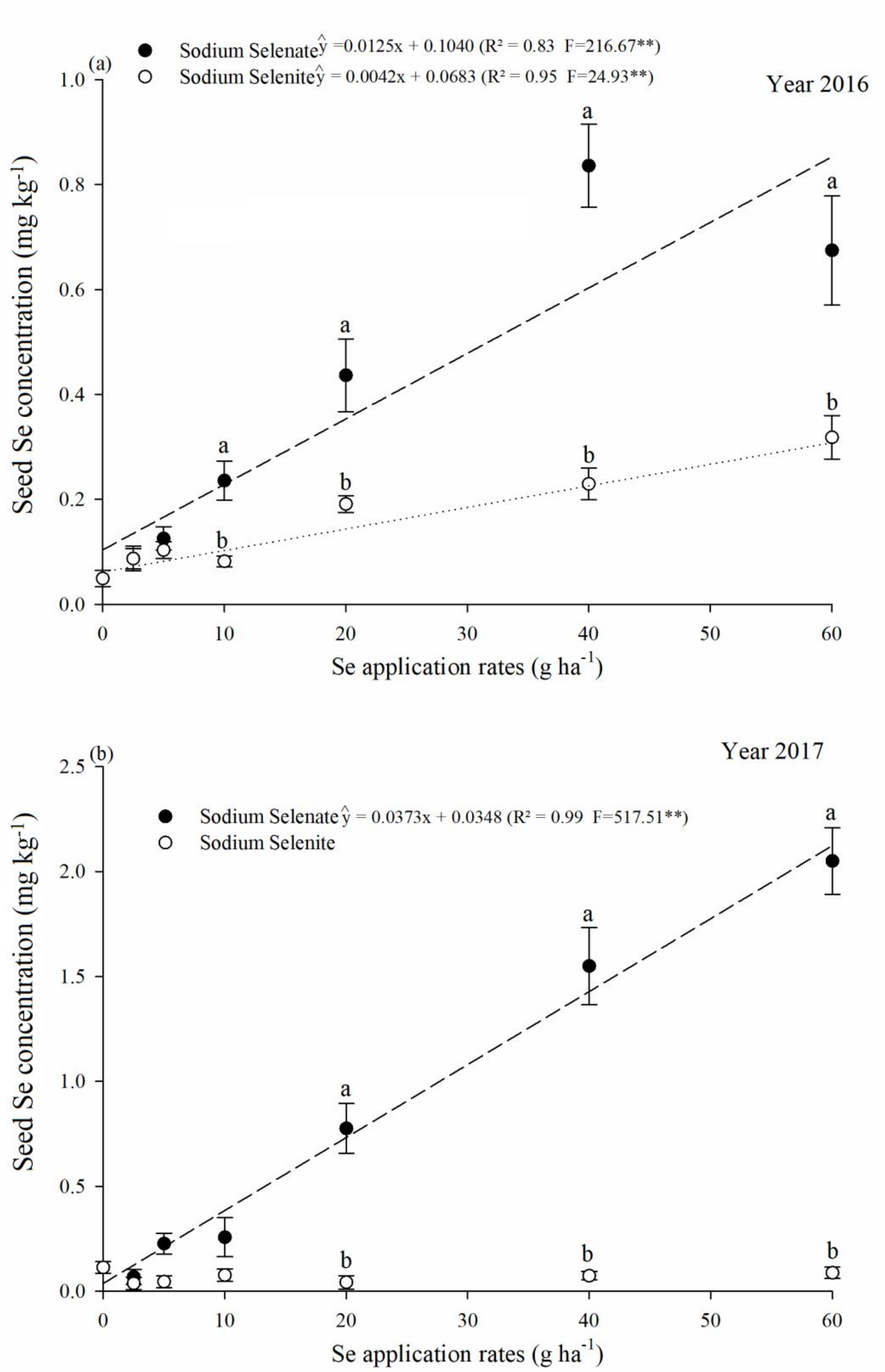


Figure 4. The effect of applying sodium selenate or sodium selenite on seed Se concentration in the first (a) and second (b) year. The standard error of the mean ($n = 4$) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0.05$). CV (%) = 37.29 (a); 42.68 (b).

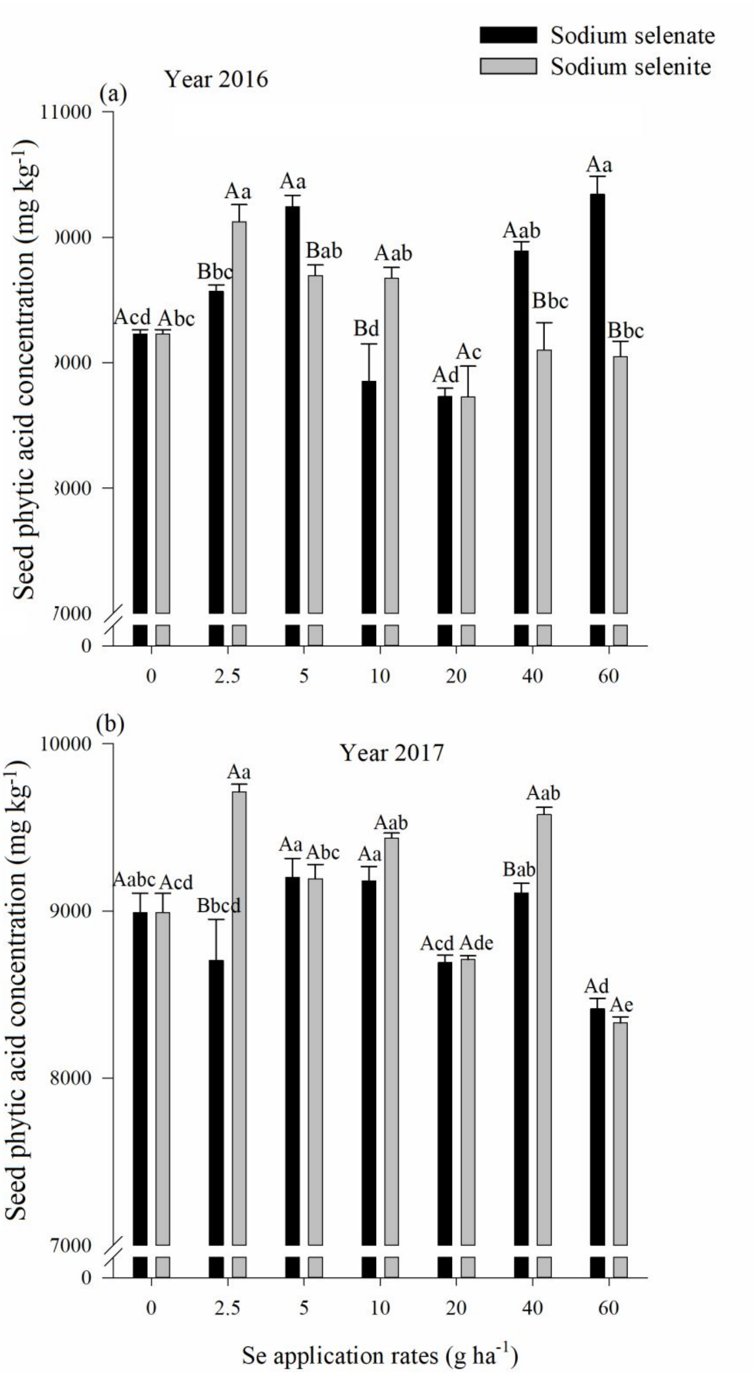


Figure 5. The effect of applying sodium selenate or sodium selenite on seed phytic acid concentration in the first (a) and second (b) year. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Se sources, and lowercase letters correspond to Se doses. CV (%) = 2.68 (a); 1.74 (b).

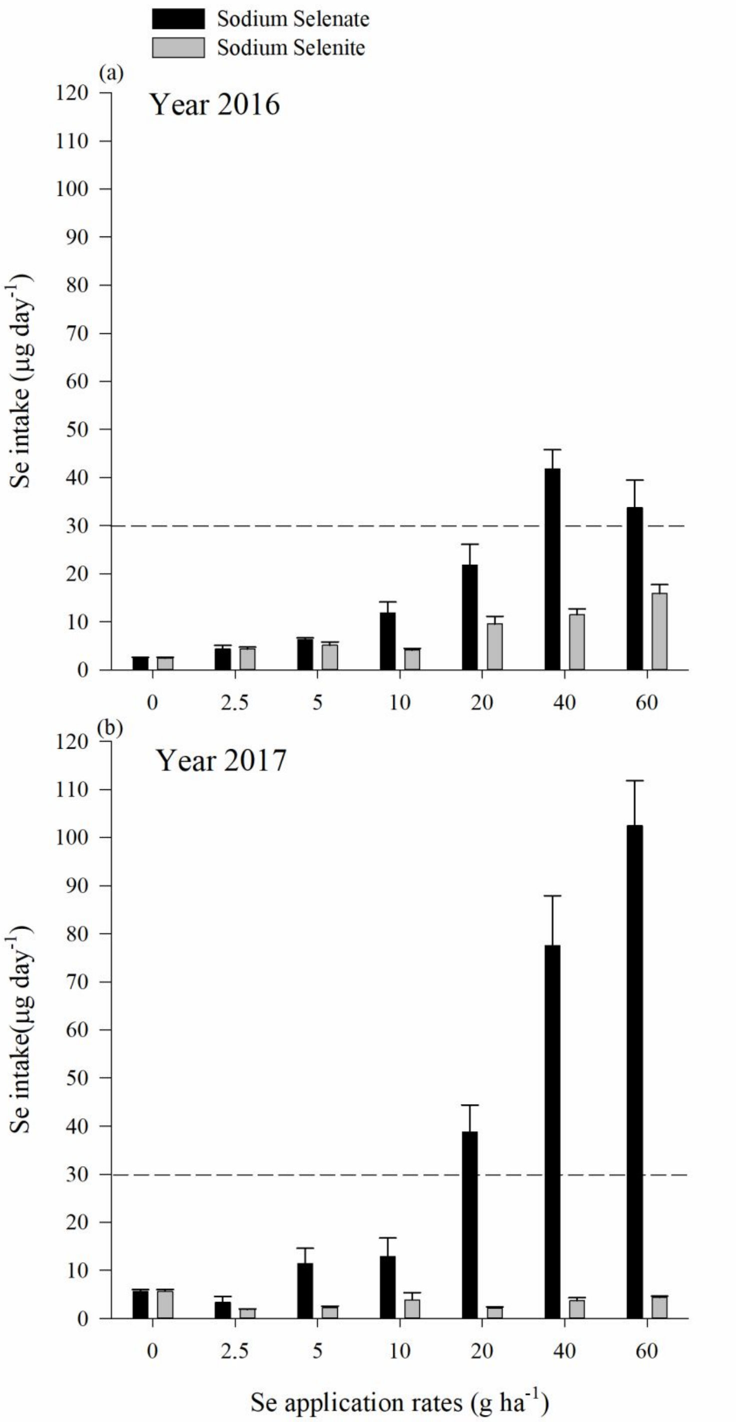
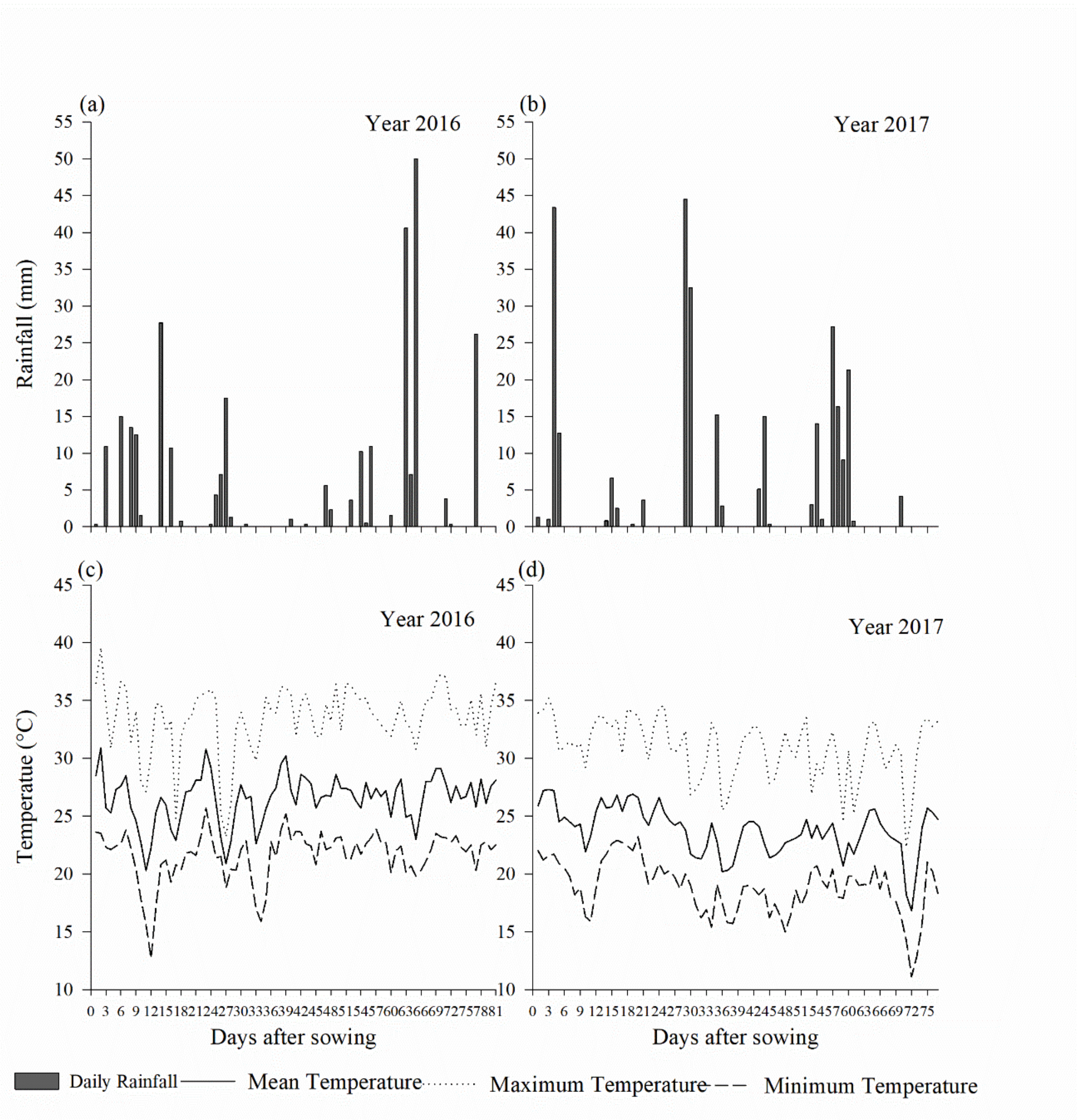
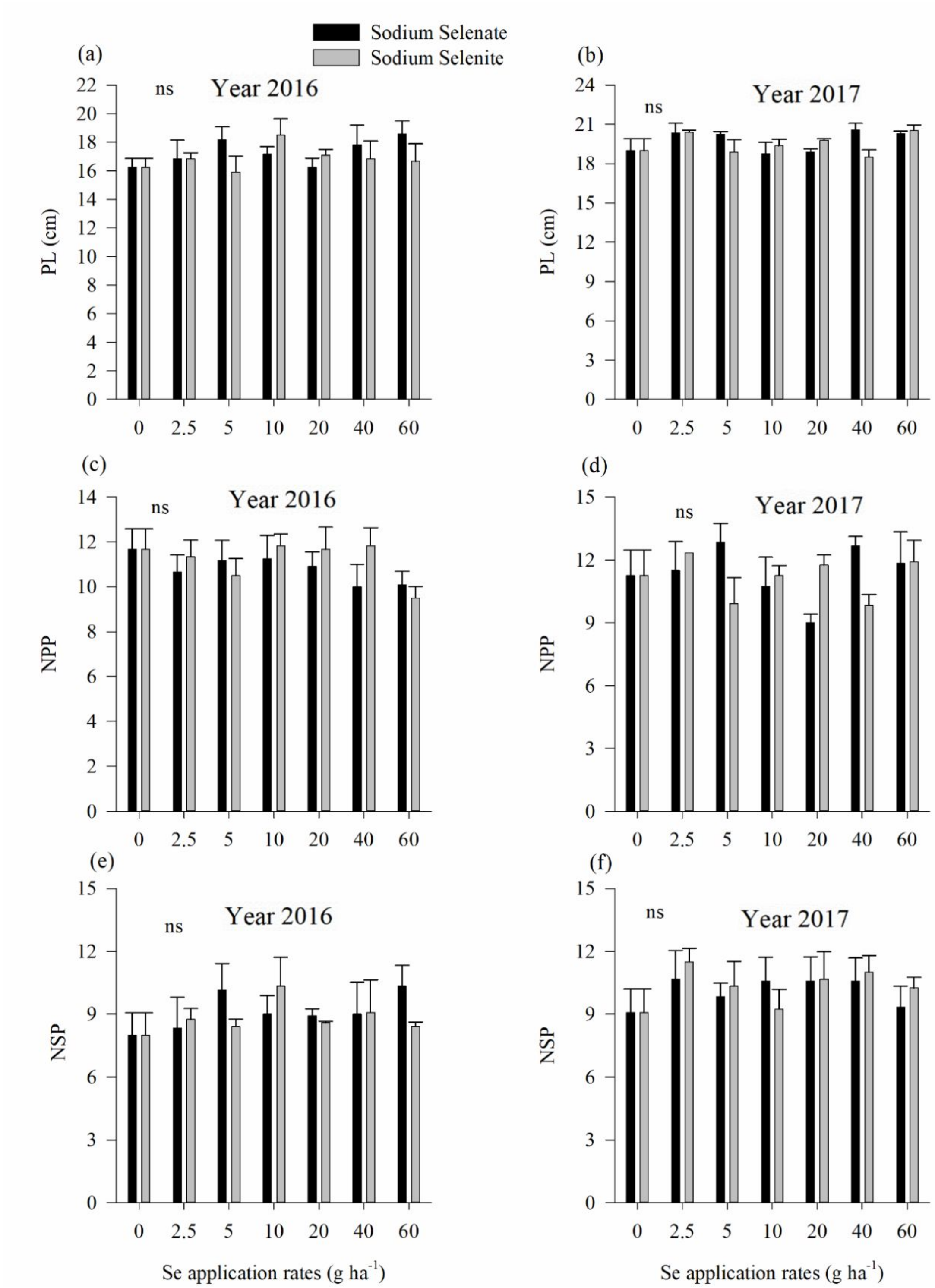


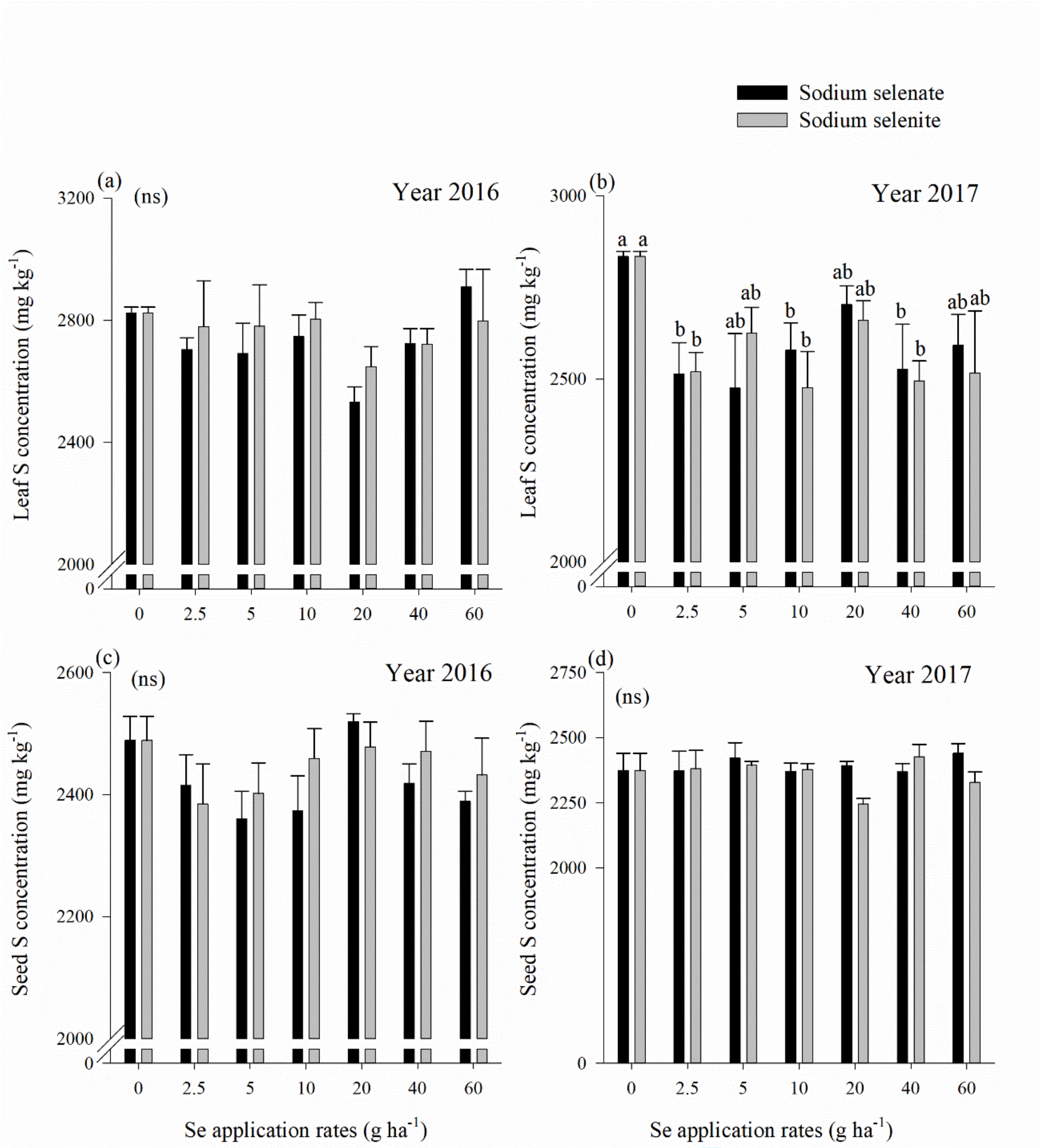
Figure 6. Daily intake of Se that would be provided by a 54.2 g portion of cowpea seeds biofortified using sodium selenate or sodium selenite in the first (a) and second (b) year of trials. Dashed black line indicates the maximum intake recommendation range.



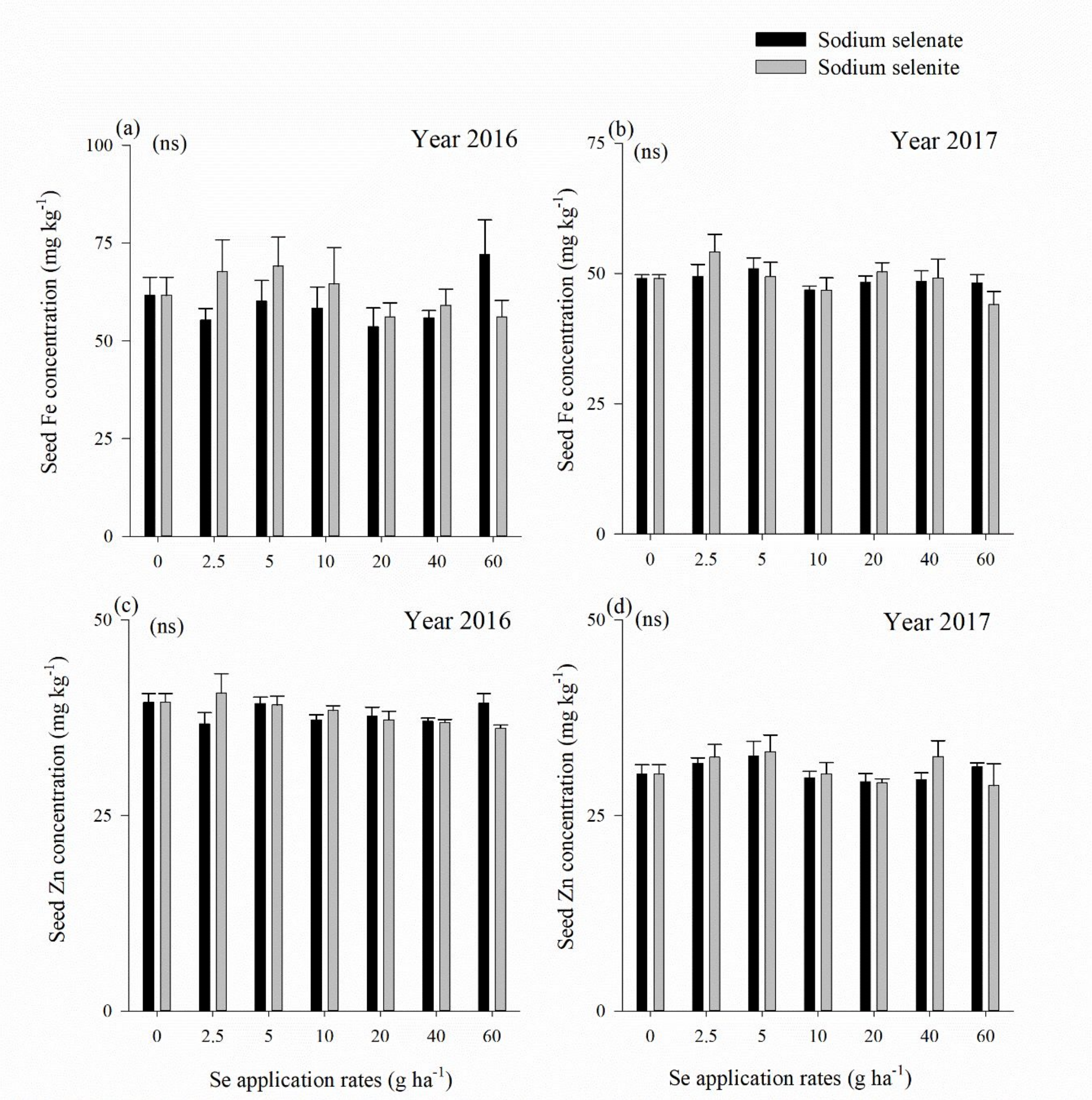
Appendix A. Rainfall (a-b) and temperature (c-d) recorded throughout cowpea field experiments.



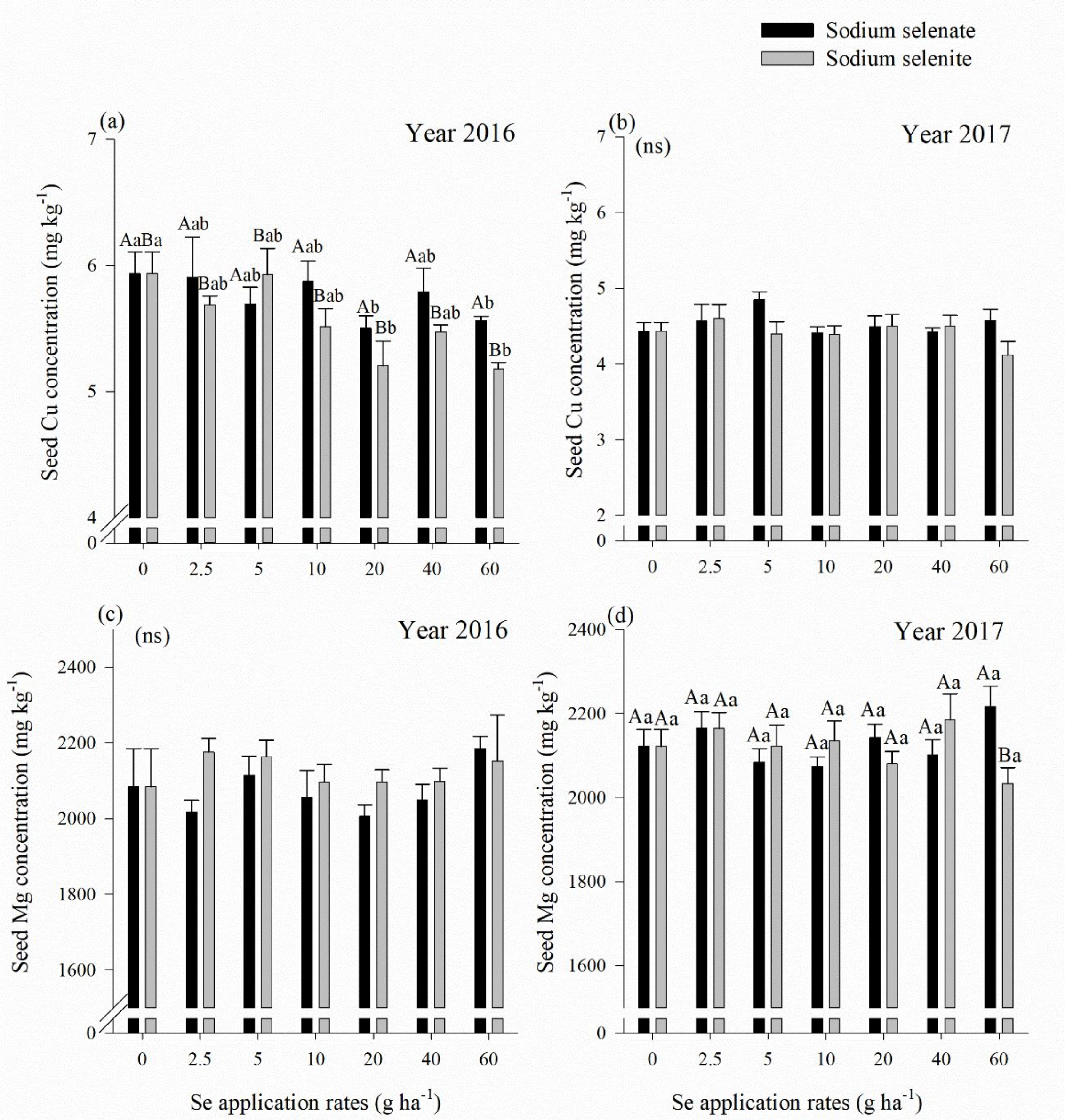
Appendix B. The effect of applying sodium selenate or sodium selenite on pod length in the first (a) and second (b) year of trials, the number of pods per plant in first (c) and second (d) year of trials and number of seeds per pod in first (e) and second (f) year of trials. The standard error of the mean (n = 4) is indicated by error bars. ns = not significant according to the Tukey test ($p \leq 0.05$). CV (%) = 11.18 (a); 6.03 (b); 15.07(c); 16.98(d); 23.33(e) and 19.33 (f).



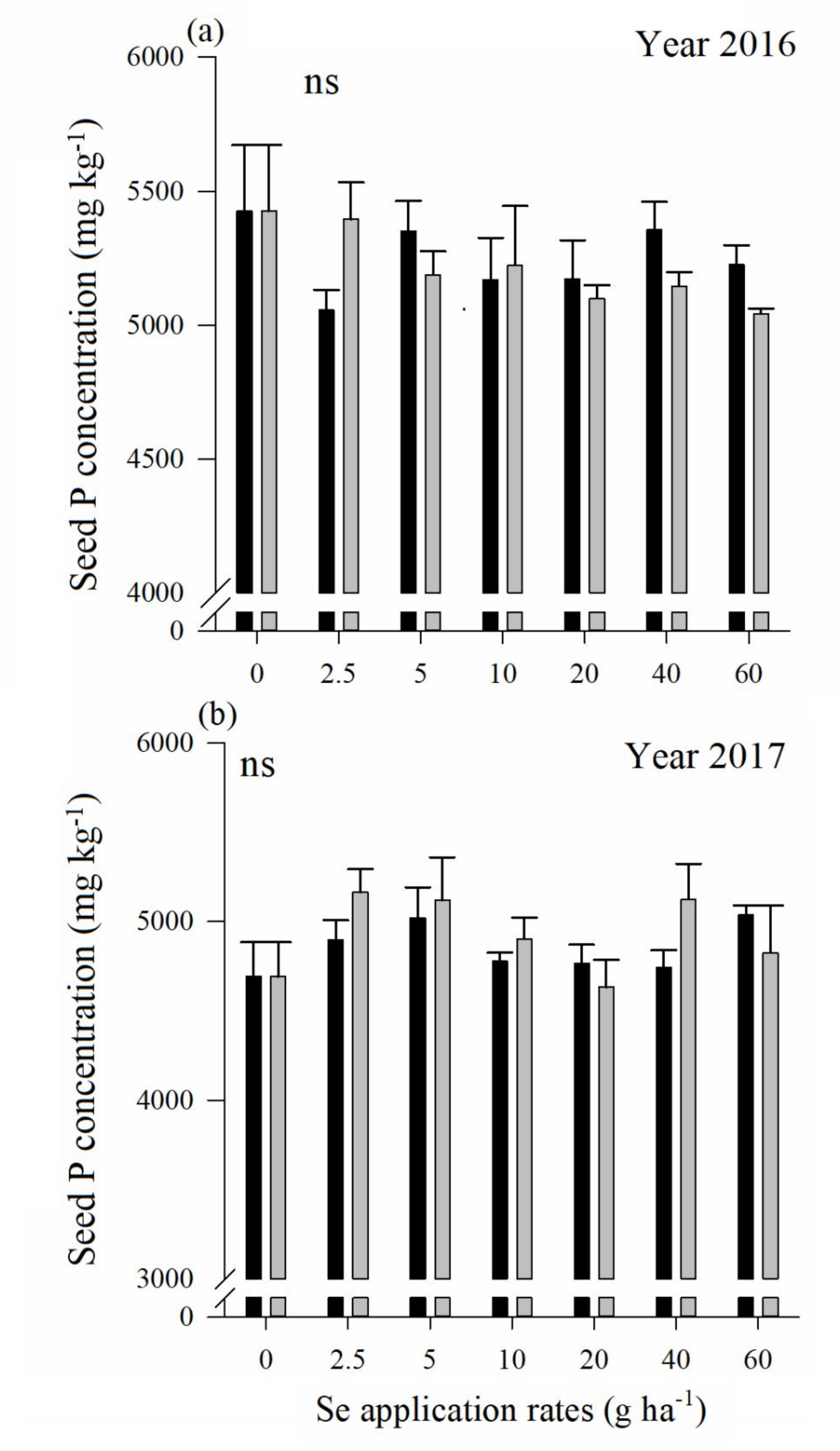
Appendix C. The effects of applying sodium selenate or sodium selenite on leaf S concentration in the first (a) and second (b) year and seed S concentration in the first (c) and second (d) year. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Se sources, and lowercase letters correspond to Se application rates. ns = not significant according to a Tukey test ($p \leq 0.05$). CV (%) = 6.46 (a); 7.11 (b); 3.84 (c); 3.60 (d). Se application rates effect regardless of sources according to a Tukey test ($p \leq 0.05$) observed in: b.



Appendix D. The effects of applying sodium selenate or sodium selenite on seed Fe concentration in the first (a) and second (b) year and seed Zn concentration in the first (c) and second (d) year. The standard error of the mean (n = 4) is indicated by error bars. ns = not significant according to a Tukey test ($p \leq 0.05$). CV (%) = 17.14 (a); 8.64 (b); 5.64 (c); 9.27 (d).



Appendix E. The effects of applying sodium selenate or sodium selenite on seed Cu concentration in the first (a) and second (b) year and seed Mg concentration in the first (c) and second (d) year. The standard error of the mean (n = 4) is indicated by error bars. Different letters indicate difference between means according to a Tukey test ($p \leq 0.05$). Uppercase letters correspond to Se sources, and lowercase letters correspond to Se application rates. ns = not significant according to a Tukey test ($p \leq 0.05$). CV (%) = 5.65 (a); 9.27 (b); 5.79 (c); 3.67 (d).



Appendix F. The effects of applying sodium selenate or sodium selenite on seed P concentration in the first (a) and second (b) year. The standard error of the mean (n = 4) is indicated by error bars. ns = not significant according to a Tukey test ($p \leq 0.05$). CV (%) = 4.53 (a); 6.45 (b).