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Study on the relationship between soil selenium and plant selenium uptake

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Abstract

Various extraction methods have been used to determine selenium (Se) concentrations in soils and plants in the second seleniferous regions of China. Our results show tea Se contents in the study area range from 1.009 to 2.6 mg/kg, which reveal that the tea areas in Ziyang County are in seleniferous regions. The four extraction methods evaluated in this study provide different information concerning soil and plant Se levels. The quality control/quality assurance program for this project indicated there is excellent agreement between total soil Se and extractable Se. For example, phosphate extractable Se results from the field investigation and greenhouse study were found to be highly correlated ($R^2 > 0.91$) by linear regression analyses. Results from rye seedling experiments further show phosphate extractable Se has significant correlations with plant Se uptake and that a 0.1 M solution of KH_2PO_4 can be used as the extractant of soil available Se. In the acid soil, the *Brassica campestris* yield could be significantly reduced when the content of Se^{6+}–Se ≥ 0.5 mg/kg, and the influence on the yield was not as obvious when the content of Se^{6+}–Se reached up to 2.0 mg/kg. The uptake by *Brassica campestris* of Se^{6+}–Se is higher than that of Se^{4+}–Se. The main factors influencing the biological availability of soil Se, in order of their importance are $CaCO_3$, the presence of silt grains, organic matter and the presence of clay grains. pH could affect KH_2PO_4 extractable Se through $CaCO_3$.

Introduction

Selenium (Se) is a naturally occurring trace element that is vital to life and occurs essentially in all soils and biological materials with quantities varying from 0.005 parts per million (ppm) in a soil-deficient area of Finland to 8000 ppm in the Tuva area of Russia (Berrow and Ure, 1989). Selenium in the human diet is acquired from nutritional sources, the amount of selenium available in foods being dependent on the species and the soil selenium content. However, certain areas of many countries (Australia, Canada, China, Russia, UK, US) have selenium-deficient soils, while other areas have selenium-toxic soils (Orrille, 1964; Berrow and Ure, 1989). High concentrations of selenium occur naturally in some soils, especially those derived from Cretaceous shale parent materials (Terry and Zayed, 1998). Both high and low concentration extremes are troublesome. Selenium deficient soils could lead to selenium deficient diets caused Keshan disease in most Chinese northeastern area; too much selenium could lead to selenium toxication caused livestock “alkali disease” (such as blind staggers in affected cattle) and Se poisoning symptom in humans such as lose hair, skin decolor, nail abnormity and anesthesia (Yang, 1989).

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Several areas within central western China are known to contain high levels of selenium in soil and geological formations (Chen et al., 1980; Li, 1980; Mei, 1980). In the study on the etiology of an endemic disease characterized by loss of nails and hair in Enshi County, (Yang, 1981) reported the seleniferous sedimentary materials were found contain the greatest geological source of Se in Enshi County of Hubei Province and Se concentrations range from <1 to over 500 mg Se/kg (ppm); the highest levels are typically found in Cretaceous black shales. The high levels of selenium in soil and vegetation were also found and the livestock "alkali disease" and the etiology of an endemic disease characterized by loss of nails and hair in humans were investigated in these areas. Then the Enshi County was named the first Chinese Seleniferous area. In 1984, the Ziyang County located the Shaanxi Province were also found the phenomena similar to the Enshi County then named the Second Chinese Seleniferous area in China (Chen, 1984; Zhou, 1984). These areas could develop into potential problem spots for wildlife and livestock if high Se-containing vegetation (>5.0 μg/g Se) growing on these sites is consumed over time. Areas adjacent to seleniferous regions may also be affected if Se is translocated to surface or ground water through wind or water movement. The consequences of this may be increased Se availability to plants, which could result in greater plant Se uptake and the possibility of Se toxicity to animals (Chen, 1984; Oldfield, 1998) and increased wildlife concerns in reclaimed environments (Boon, 1989; Sharmasarkar and Vance, 1995a, b).

Selenium, being a naturally occurring trace element that is vital to life, could cause various pathological changes in people and animals if the Se content in their foods is too low or too high (Rosenfeld, 1964; Shamberger, 1983). It is necessary to study the plant Se uptake and high Se-containing vegetation owing to Se entering the food chains of people and animals through cereals, vegetables and herbage. Some results show that Se absorption by crops for growth relies on oxidated forms of Se. Contrasting additions of Se^{4+}–Se and Se^{6+}–Se added to soil, showed that plants absorb Se in high concentration in Se^{6+}–Se added soil (Ganje, 1958). The availability of Se^{4+}-Se added into soil was relatively low as it is absorbed by hydrate oxide or degraded into elemental Se or selenide (Cary and Nielsen, 1973). Selenium could restrict soybean growth in Se added soil, and the degree of restricted growth is determined by the forms of the added Se, as expressed by the following gradient: $SeO_4^{2-} > HSeO_3^- > SeO_3^{2-} > Se^0$ (Singh and Singh, 1978). From this, it is clear that Se in the environment is not completely absorbed by plants; only those in biologically available forms could enter plants.

The relationship between soil Se and plant Se uptake is not well understood. Information from greenhouse and growth chamber studies (Jump and Sabey, 1985; Wan et al., 1988; Wanek et al., 1999) has shown better correlations than field studies (Williams and Thomton, 1973; Sharmasarkar and Vance, 1995a). With greenhouse and growth chamber studies, experimental conditions were usually controlled (i.e., using similar soil and plant materials, regulating light, water and temperature), in order to reduce study variability. Factors and conditions of field studies may vary both spatially and temporally (Wan et al., 1988; Prodgers and Munshower, 1991; Borowska and Koper, 2000). A partial list of different Se extraction methods used to correlate soil-plant Se, and the study conditions (i.e., environmental and vegetative) reported in the literature are presented in Table 1.

Research is needed to define the relationship between total soil Se and available soil Se for plant uptake. Essentially, we need field studies that investigate possible interactions between selenium and other soil physicochemical properties over sites having diverse plant communities. At the same time, we need to improve on methods for sampling, handling, preservation, extraction and quantification of soil and plant materials. We have to develop extraction methods that provide meaningful representations of plant-available soil selenium that, above all, give consistent values. This study attempted to provide information on the variability of soil selenium determined by different methodologies and the factors influencing soil physiochemical properties. The objectives of this study were to: (1) examine directly and rapidly the biological availability of soil Se with a rye seedling method; (2) test the influence of selenium in two oxidation states (Se^{6+}–Se and Se^{4+}–Se) added to an acid soil on the growth of black cabbage (*Brassica*

Table 1. A partial list of the different extraction methods, study conditions and vegetation species used in various Se studies

Method	Study	Plant	Reference
Water or Hot Water Soluble	Field	Western wheatgrass	Olson et al., 1942
	Greenhouse	*Perennial ryegrass*	Williams and Thomton, 1973
	Greenhouse-Se amended soils	*Alfalfa*	Soltanpour and Workman, 1980
	Greenhouse-high Se materials	*Saltbush & Astragalus bisulcatus Grasses*	Jump and Sabey, 1989
	Field	*Forbs, Shrubs*	Sharmasarkar and Vance, 1995a
AB-DTPA	Greenhouse-Se amended soils	*Alfalfa*	Soltanpour and Workman, 1980
	Greenhouse	*Saltbush & Astragalus bisulcatus*	Jump and Sabey, 1989
	Field	*Greasewood*	Jump and Sabey, 1985
	Field	*Winter wheat*	Soltanpour et al., 1982
	Field	*Variety of Plants*	Prodgers and Munshower, 1991
	Field	*Grasses, Forbs, Shrubs*	Sharmasarkar and Vance, 1995a
Base Soluble	Field and Greenhouse	*Western wheatgrass*	Olson et al., 1942
	Field and Greenhouse	*Perennial ryegrass*	Williams and Thomton, 1973
DTPA or EDTA	Greenhouse	*Saltbush & Astragalus bisulcatus*	Jump and Sabey, 1989
	Greenhouse	*Perennial ryegrass*	Williams and Thomton, 1973
Phosphate	Field	*Grasses and Forbs*	Vance, 2000
	Field	*Grasses, Forbs, Shrubs*	Sharmasarkar and Vance, 1995a
Saturated Paste	Greenhouse	*Saltbush & Astragalus bisulcatus*	Jump and Sabey, 1989
	Field	*Grasses, Forbs, Shrubs*	Sharmasarkar and Vance, 1995a
	Greenhouse	*Yellow sweet clover & Fourwing saltbush*	Wanek et al., 1999

campestris) and its absorption through a potted plant experiment; and (3) research the relationship between the soil available Se and plant Se uptake by combining field experiments.

Materials and methods

Field investigation

According to the geographical distribution of soil selenium in the second seleniferous region in Ziyang County, Shaanxi Province, China, the 55 soil samples were divided into two types: one includes 46 cultivating topsoil samples; another includes 9 section soil samples from the 3 typical soil profiles, which were sampled based on their occurring hierarchies. After the soil samples were dried, the fine roots and sundries were removed, and all soils were passed through nylon screens of 1 mm and 0.1 mm in aperture respectively. The former was used for extractable Se measurements, and the latter to measure total soil Se.

Ten samples of spring tealeaves (a mixture of first buds, second and third leaves) were collected while sampling soil. All tealeaves were washed two times with deionized water after washing with a detergent of 0.1% in concentration. All plant samples were dried at 55 ± 5 °C, milled and mixed, then passed through a nylon screen of 1 mm in aperture. Then, the mixture was put into a jar for total Se measurement and the soil properties such as pH, CEC, organic matter content, soil particle composition, available iron and $CaCO_3$ content, were measured according to the routine analytical methods (Page, 1982; Reuter, 1996).

Pot experiment

A main objective of this experiment was to assess the use of added selenium in determining its availability and toxicity to plants. In the individual experiment, six treatments were arranged based on the complete random area and each treatment were duplicated four times. In the five treatments (I, II, III, IV, V), the ratios of Se^{6+}–Se and Se^{4+}–Se were

2.0:0, 1.5:0.5, 1.0:1.0, 0.5:1.5 and 0:2.0, respectively, so as to keep the total Se content of each treatment at 2.0 mg/kg level. Moreover, the blank treatment was arranged. Soils were sampled in the second seleniferous regions in Ziyang County.

Each pot of 13 × 13 cm in size, was filled with 1.5 kg of soil and 0.23, 0.10 and 0.10 g/kg soil of nitrogenous, phosphate and potash fertilizers respectively, were used in the experiment. Black cabbage (with a germination rate of 98%) was planted in each pot. The final seedlings (20 seedlings in each pot) were done after the buds put forth for 2 weeks. The black cabbage was irrigated with deionized water during the whole growth stage. The ground biomass was weighed after 7 weeks. The fresh stems were cut and dried for 15 min under temperature of 80–90 °C to kill active enzymes, then dried under a constant temperature of 55 °C and were grinding for the total Se measurement.

Rye seedling experiment

The rye seedling (*Secale cereale* L.) method was first put forward by Neubauer and Schneider 1923 and used to measure rapidly the availability of nutrient elements in soils.

The experimental vessels include beakers of 1000 ml and glass tubes of 6 × 70 mm used for watering. Quartz sand used in the experiment was 0.6–2.0 mm in grain size. It was dipped in HCl of 1 M for one day and churned several times during marinating. After the HCl was poured out, the sand was washed with deionized water until it contained no Cl^-, and dried at a temperature of 105 °C.

The rye seeds used in the experiment (the germination rate to be higher than 95%) were air dried on a piece of filter paper. A piece of gauze was spread in a tray and soaked with water, prior to the seeds being placed on it one day before the experiment. The tray was put into a growth chamber for germination under temperature of 20 °C (for about 10 h).

Then, each of the 100 g samples of the 12 soils was passed through a screen of 1 mm in aperture and were fully mixed with 50 g quartz sand, and spread in a tray. A glass tube was inserted into the mixture for watering. Then, the rye seeds were equally put on the mixture and pressed, covered with 250 g quartz sand and poured with deionized water. The beaker was covered with a covering utensil which was propped up with matchsticks. The installation of the contrast trays without soil were constructed in the same method mentioned above, with only 150 g of quartz sand put into it. The experiment was carried out in triplicate.

The trays were put into a greenhouse under a regulation temperature of 20 ± 2 °C, irradiated with daylight lamps all day, and kept away from direct sunshine. The lost water was supplemented with a burette through the glass tube after weighting everyday. Water supplements were added twice a day in the later stage of the experiment, and the location of the pots was changed everyday so as to allow uniform illumination.

After 14 days, the seedlings with soil-sand were removed from the pots and washed with deionized water in order to remove soil and sand, then put on a tray and cut the roots. The roots and the ground biomass were separated and dried at a temperature of 60 °C, then sieved with a nylon screen with an aperture of 1 mm.

Selenium analysis

Several extractable methods have been proposed for correlating soil Se concentrations with plant uptake. Soil Se has been characterized by extractable techniques such as hot water, AB-DTPA, DTPA, EDTA, Na_2CO_3, saturated paste and phosphate extracts, as well as total Se levels (Jump and Sabey, 1989; see Table 1). Firstly, soil and plant samples were analyzed for total Se (after acid digestion), then the hot water, 0.25 M KCI, 0.5 M $NaHCO_3$ and 0.1 M KH_2PO_4 were used sequentially to extract soil Se. The four extraction methods have been suggested to represent plant available Se (Olson et al., 1942; Spackman et al., 1980, 1994; Vance, 2000; Williams and Thompton, 1973). In order to valid any one-extraction method for evaluating plant Se uptake, it must provide a comparable, reproducible and representative extractable Se which has high correlationship with plant uptake. In this studies the three soils (see Tables 2 and 3) were also selected to valid the impact of extractable times on the different soil Se keeping 0.1 M KH_2PO_4 concentrations constant and the impact of KH_2PO_4 concentrations on the different soil Se keeping extraction time constant (2 h). Then selenium in each extraction method

Table 2. Impact of extractable times on the different soil Se keeping 0.1 M KH_2PO_4 concentrations constant

Extractable time (h)	Extractable Se content (mg/L)		
	Soil 1	Soil 2	Soil 3
	Average value ± standard deviation		
1	0.050 ± 0.004	0.111 ± 0.003	0.321 ± 0.007
2	0.055 ± 0.003	0.118 ± 0.004	0.332 ± 0.005
4	0.058 ± 0.005	0.119 ± 0.007	0.339 ± 0.009
8	0.065 ± 0.004	0.129 ± 0.005	0.341 ± 0.004
16	0.065 ± 0.003	0.132 ± 0.008	0.346 ± 0.005
24	0.064 ± 0.005	0.133 ± 0.006	0.349 ± 0.007

Table 3. Impact of KH_2PO_4 concentrations on the different soil Se keeping extraction time constant (2 h)

Extractable concentration (mg/l)	Extractable Se content (mg/L)		
	Soil 1	Soil 2	Soil 3
	Average value ± standard deviation		
0.05	0.042 ± 0.004	0.109 ± 0.007	0.324 ± 0.010
0.1	0.055 ± 0.005	0.118 ± 0.004	0.332 ± 0.005
0.2	0.064 ± 0.002	0.121 ± 0.009	0.338 ± 0.004

was analyzed by diaminonaphthalene fluorometric method (Page, 1982; Reuter, 1996).

Multivariate analysis of among soil Se, extractable Se, plant se and soil factors was done by SPSS software and statistical diagnostics (Penny, 1996).

Results and discussion

Soil Se and extractable Se

The Se availability of soil to plants can be verified by the plant Se uptake. Se existed in soil in the different forms including selenate (Se^{6+}), selenite (Se^{4+}), elemental selenium (Se^0), selenide (H_2Se), organic selenide, etc., in which the selenate, partial selenite and organic selenide could be available for plant uptake. As yet, the bioavailable Se in its different forms has been difficult to separate and identify due to the extremely complex physiochemical properties of soil (Davies, 1980). Currently, the Se in an aqua-soluble form was regarded as the soil available Se, however, it is difficult to measure in selenium deficient soils due to the weak buffer capability of the aqueous solution, easy change of pH and weak extraction capability of water. On the contrary, the content of extractable Se with water from high Se level soils was not so high. For example, the total Se content of soil was 23.53 mg/kg, but the water extractable Se was 56.38 μg/kg in the Se-toxic soils of Ziyang County, which occupy only 0.24% of the total soil Se. In fact, it was considered according to the practical conditions that the extractable Se could be used to reflect the Se availability for plants in seleniferous soils. For these reasons, water, 0.5 M $NaHCO_3$, 0.25 M KCl and 0.1 M KH_2PO_4 were separately used as extractants to extract the available Se for further comparisons.

The four extraction methods used sequentially to extract soil Se in this study provide different information concerning total soil Se and extractable soil Se. The analytical results were shown that the total soil Se and total extractable Se has high linear relationship and the correlation coefficient is greater than 0.95 (Figure 1). As comparing the relationship among various soil extractable Se and total soil Se concentrations by multivariate analysis, several trends were found if using a correlation coefficient of 0.9 or greater.

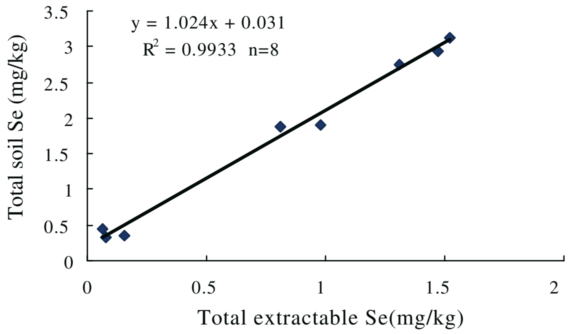

Figure 1. The correlationship between total soil Se (Y) and total extractable Se (X).

A consistent relationship was found which indicated water, $NaHCO_3$, KCl and KH_2PO_4 extractable Se were all statistically correlated. And all correlation coefficients for the eight soils sampled, were less than 0.95. By further multi-gradual regressive analysis, the result indicated that only KCl extractable Se and KH_2PO_4 extractable Se remained, then the obtained regressive equation was

$$Y = 0.2198 + 0.0153X_3 + 1.0587X_4 \quad (R = 0.9735, \mathrm{n} = 8), \tag{1}$$

where, Y is total soil Se, X_3, X_4 is KCl extractable Se and KH_2PO_4 extractable Se respectively, and the multiple correlative coefficient, R, was highly significant. From Equation (1), the trend was found that KH_2PO_4 extractable Se presented the most contribution to the total soil Se and KCl Se was second. The KH_2PO_4 extraction, however, may better estimate the Se concentration related to plant uptake and mobility in soil. This indicated that KH_2PO_4 extractable Se may be solubilized readily by plant–root interactions and approximate the amount of Se concentration by plant uptake.

The chemistry of Se is, in many respects, similar to S chemistry. Thus, one might conclude that an extractant containing SO_4^{2-} may be useful for extracting Se. A study by Cary et al. (1967) found Se extracted from soils with K_2SO_4 was related to Se in alfalfa. Selenium extracted by K_2SO_4 is due to dissolution and exchange reactions involving the SO_4^{2-} ion. A problem associated with using K_2SO_4 is that many soils in arid and semiarid environments already contain very high levels of SO_4^{2-}. But in humid environments, a method used widely for determining soil extractable SO_4^{2-} utilizes solutions containing phosphate (PO_4^{3-}). This would represent a logical choice of an extractant to determine extractable or plant-available Se. Phosphate-extractable Se would include both water soluble and adsorbed Se species. The phosphate ion would displace the divalent Se species from adsorption or complexion sites. The present study also suggests that phosphate-extractable Se is less variable than Se determined by water, $NaHCO_3$, KCl extractions (Spackman et al., 1994).

Due to the lack of consistency in methods for accurately determining soil available Se and high extractable Se concentrations, an emphasis should be placed on the development of better analytical techniques so that the results of Se analysis can be used with confidence. For that reason, time and concentration of KH_2PO_4 extractions were further tested in this study. Results of the extraction analysis proved that a suitable time for the extraction process is two hours and the economic extractable concentration of KH_2PO_4 is 0.1 M (see Tables 2 and 3).

Se uptake by tea

Tea Se contents in the study area ranged from 1.0 to 2.6 mg/kg, which shown that Ziyang County is the seleniferous area (Table 4).

The statistical diagnostics results show the Se uptake by plants has no significant correlation with the water extractable Se and $NaHCO_3$ extractable Se, but a significant correlation with KCl extractable Se (x_3) and KH_2PO_4 extractable Se (x_4). Using the Se content in plant bodies (y) and various extractable Se, the result by a multi-gradual regressive analysis indicated that

Table 4. Relationship between tea Se and various extractable Se

Item		H_2O–Se X_1	$NaHCO_3$–Se X_2	KCl–Se X_3	KH_2PO_4–Se X_4	Plant–Se Y
X_1	H_2O–Se	1				
X_2	$NaHCO_3$–Se	0.6944	1			
X_3	KCl–Se	0.8313	0.7622	1		
X_4	KH_2PO_4–Se	0.8308	0.7274	0.9023	1	
Y	Plant–Se	0.8645	0.7057	0.9409**	0.9544**	1

$\alpha_{0.01} = 0.92$.

only KH_2PO_4 extractable Se remained. The obtained regressive equation was:

$$y = -4.25 + 0.063x_4 \ (R = 0.9108, n = 16) \tag{2}$$

and the multiple correlative coefficient, R, was significant to a level of 0.05, which reveals that only the KH_2PO_4 extractable Se (x_4) reflects the Se availability to plants.

Se uptake by rye seedlings

The soil Se contents, distribution law and extraction available Se were studied in main soils in Ziyang County, Shaaxi Province. The rye seedling experiment was also carried out by 12 soils coming from seleniferous area. Results are shown in Table 5.

Using a correlation coefficient of 0.9 or greater, the Se content of rye seedling roots is higher than that in their leaves, the Se content of rye seedlings roots (y_1) and leaves Se (y_2) and the total Se ($y_3 = y_1 + y_2$) have no correlation with the hot water extractable Se (x_1), 0.1 M KH_2PO_4 extractable Se (x_2), and 0.5 M $NaHCO_3$ extractable Se (x_3). However, the product of the total Se concentration (y_3) times the total dry weight of the seedlings (W) has a significant correlation with x_2, but has no correlation with x_1 and x_3. The multi-regression equation is:

$$y_3 \times W = 0.31004 + 0.01975x_2 \ (R = 0.9537, n = 12) \tag{3}$$

Thus, the solution of 0.1 M KH_2PO_4 was recommended as the extractant for extracting the biological available Se in acid and seleniferous soil.

Black cabbage Se and soil Se

Se uptake by black cabbage

The pot experimental results are shown in Table 6. In treatments I, II and III, the stems of black cabbage in its seedling stage were turning yellow, there were a few brown spots on the leaves, and the leaf edges were purple. In treatments II, and I the black cabbage was in its

Table 5. The Se uptake by rye seedlings and soil Se in Ziyang County

Type of soils	1	2	3	4	5	6	7	8	9	10	11	12
Se uptake by Rye seedling												
Total soil Se (mg/kg)	23.53	17.05	5.95	2.76	1.85	0.45	1.49	1.88	1.99	2.93	1.36	0.37
Dry yield (g) (W)	4.37	3.64	2.02	2.58	2.43	2.24	2.69	2.74	2.56	2.44	2.39	2.65
Leaf & shoot Se (mg/kg)(Y_1)	0.55	0.12	0.10	0.16	0.07	0.06	0.07	0.10	0.08	0.06	0.02	0.03
Root Se (mg/kg) (Y_2)	1.86	0.79	0.27	0.64	0.48	0.04	0.57	0.70	0.43	0.71	0.08	0.11
Total Se (mg/kg) (Y_3)	2.41	0.90	0.37	0.80	0.56	0.10	0.64	0.80	0.51	0.77	0.10	0.14
$W \times Y_3$ (mg)	10.51	3.29	0.74	2.07	1.91	0.22	1.72	2.18	1.31	1.87	0.24	0.37
Extractable Se (μg/kg)												
H_2O-Se(X_1)	56.38	13.03	4.71	24.37	4.21	1.12	5.83	23.15	15.67	11.68	9.92	2.21
KH_2PO_4–Se (X_2)	331.80	209.70	64.58	82.19	49.11	5.99	54.50	69.00	63.35	41.31	63.38	60.76
$NaHCO_3$–Se (X_3)	157.20	97.62	38.75	75.57	29.09	6.81	74.87	79.45	63.38	19.12	59.56	31.06

Table 6. The Se uptake by black cabbage and soil Se in Ziyang County

No. of treatment	Blank	I	II	III	IV	V
Se^{6+}–Se:Se^{4+}–Se	0.0:0.0	2.0:0.0	1.5:0.5	1.0:1.0	0.5:1.5	0.0:2.0
Added Se (mg/pot) Se^{6+}:Se^{4+}	0.0:0.0	3.0:0.0	2.25:0.75	1.50:1.50	0.75:2.25	0.0:3.0
Dry yield (g/pot)	10.90	2.72	5.80	8.52	10.11	11.32
Se content in black cole (g/kg)	0.01	0.31	0.23	0.18	0.09	0.03
Analysis on Se balance						
Dry yield*Se content (mg/pot)	0.09	0.83	1.33	1.54	0.93	0.35
Residual Se in soil (mg/pot)	0.45	1.69	1.20	0.87	1.48	2.00
Reclaimed ratio (%)		83.90	84.40	80.47	80.33	78.33

mid-growing stage, the leaf tines became white, which was possibly caused by the over accumulation of Se in the stem bodies. The abnormity did not occur in other treatments.

The influence of Se on the black cabbage yield changed asymptotically with the addition of Se in the different oxidation forms. But this change was not as clear when contrasting the content of Se^{6+}–Se above and below 0.5 mg/kg. The black cabbage yield was reduced as the content of Se^{6+}–Se was over 0.5 mg/kg. This reveals that Se-toxicity on black cabbage occurred only when the content of Se^{6+}–Se was over 0.5 mg/kg.

The difference between the various treatment yields confuses the relationship between the Se availability and the added Se forms. Table 6 shows the yield was significantly increased as the quantity of added Se^{4+}–Se was increased; the Se content in black cole was significantly reduced. In order to avoid the influence of yield difference, the Se uptake by black cabbage was calculated by using the dried yield and the Se content of black cabbage. The results further indicated the Se^{6+}–Se were more available to black cabbage than Se^{4+}–Se.

Analysis on the budge of the added Se

In order to further research of Se uptake by black cabbage, the contents of the initially added Se, residual soil Se after harvest and black cabbage Se uptake was also analyzed (Table 6). The Se budget in the experiment was analyzed by taking the ratio of the summation of the residual soil Se after harvest and black cabbage Se uptake and the initially added Se as the reclaimed quantity so as to further explain the availability of Se.

Table 6 shows the reclaimed quantity of Se was increased with an increase in the quantity of added Se^{6+}–Se, but the quantity of absorbed Se was reduced with an increase in the quantity of added Se^{4+}–Se. The specialized Se^{4+}–Se adsorption of soil clay particles can be used to explain that the lower the availability of Se and the higher content of the residual soil Se.

In all the treatments, the reclaimed rate of Se varied over a range of 78.34–84.40%, as was indicated by the difference between the added quantity and the reclaimed quantity of Se. Some Se could be present in residues of plant roots (which were not analyzed), or possibly volatilized from plants or soils. Se enters into the food chain of people and animals through cereals, vegetables and herbage, and the quantity of Se absorbed by plants was closely related to plant species. Some plants growing in the seleniferous regions could absorb and accumulate too much Se (as high as several thousand milligrams per kilogram), and generally the crops could accumulate a Se quantity less than 50 mg/kg only in their bodies (Wan, 1989).

Impact on KH_2PO_4 extractable Se by soil factors

Soil available Se was not only decided by the total Se content in soils, but also strongly affected by the soil physical and chemical properties. The all-route analysis was carried out so as to research the influence of soil factors (such as pH, exchangeable quantity, organic matter, soil particle composition, available iron and $CaCO_3$) on the KH_2PO_4 extractable Se. Based on the magnitude of correlative coefficients of the 7 soil factors to the KH_2PO_4 extractable Se, the sequence was: $CaCO_3$ (0.8148) > pH (0.4626) > silt content (−0.2936) > exchangeable quantity (−0.1648) > clay content (−0.1323) > available iron (0.1205) > organic matter (−0.0561). The direct all-route coefficients were in a sequence of $CaCO_3$ (0.9827) > silt content (0.2441) > organic matter (0.2084) > clay content (0.1746) > pH (0.1602) > available iron (−0.1598) > exchangeable quantity (−0.1438). The former reflects the close degree of all the soil factors with the KH_2PO_4 extractable Se, and the latter reflects the relatively important degree of direct influence of all the factors to the KH_2PO_4 extractable Se. The results show that $CaCO_3$ was the dominant factor affecting the soil available Se and it could adsorb Se on the surface of its nodules by recurring to the *Van der Waals* attraction. Then the silt grains, organic matter and clay grains affected strongly and directly the soil available Se, and organic matter and clay grains could adsorb Se through electrostatic attraction. pH and available iron had a positive correlation with the KH_2PO_4 extractable Se, but their all-route coefficients were negative, which reveal that the influence of pH and available iron on the soil available Se was negative. pH could affect the KH_2PO_4 extractable Se through $CaCO_3$.

In order to further explain the degree of influence of all the soil factors on available Se, the decisive coefficients of all the soil factors to soil available Se were also calculated in the all-route analysis. The decisive coefficients could more directly reflect the influence of all the soil factors to soil available Se. Based on the magnitude of all the absolute values of the decisive coefficients, the sequence was: $CaCO_3$ > silt content > organic matter > clay content > pH > iron > exchangeable quantity, and this sequence is the same with that of the all-route coefficients. It was obvious that $CaCO_3$ was the dominant factor influencing soil available Se as revealed by the direct all-route coefficients or by the decisive coefficients of single factors. The silt content, organic matter content and clay content were secondary, and the direct influence of available iron and pH was not obvious.

Conclusions

Based on the results of this study, it was recommended that 0.1 M KH_2PO_4 extractable Se is more reliable in determining soil and high Se concentrations in plant. Results of the extraction analysis proved that a suitable time for the extractable process is 2 h, and the economic extractable concentration of KH_2PO_4 extraction is 0.1 M. The phosphate extraction procedure outlined in Spackman et al. (1994) has been shown to provide useful information on soil and geological samples and for correlation to plant Se.

In the acid soil, the black cabbage yield is significantly reduced when the content of Se^{6+}–Se ≥ 0.5 mg/kg, and the influence on the yield is not obvious when the content of Se^{6+}–Se is up to 2.0 mg/kg. The uptake by black cabbage of Se^{6+}–Se is higher than that of Se^{4+}–Se.

The main factor influencing the biological availability of soil Se was $CaCO_3$, then the silt content, organic matter content and clay content. pH could affect KH_2PO_4 extractable Se through $CaCO_3$.

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