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富硒土壤中硒生物有效性的提取方法研究

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摘要 为建立适用于酸性富硒土壤有效硒提取的方法,从湖北省恩施土家族苗族自治州建始县天然富硒区采集了40份土壤样品进行水稻盆栽试验,采用连续浸提法和10种常用的单步浸提法对土壤硒形态和有效硒进行了提取、测定,并分析了不同浸提方法提取的有效硒含量与植物硒的相关性。结果显示:建始县酸性富硒土壤中水溶态和交换态硒的含量较低,分别为0.12~46.0、0.58~197.35 $\mu\text{g}/\text{kg}$;硒主要以有机结合态和残渣态等难以被植物利用的形态存在,铁锰氧化物结合态、有机结合态及残渣态硒含量分别为0.01~0.34、0.05~0.88、0.04~2.72 mg/kg ;在10种单一浸提剂中,0.5 mol/L NaHCO_3 和0.1 mol/L KH_2PO_4 溶液对土壤有效硒的提取效率较高;在不同土壤硒形态中,交换态硒与植物硒的相关性最高($r=0.80, P<0.01$),其余形态硒与植物硒含量的相关性均低于交换态硒。在单一浸提法中,0.1 mol/L KH_2PO_4 溶液和0.5 mol/L NaHCO_3 溶液提取的土壤有效硒含量较高,但前者与植物硒含量的相关性更为显著($r=0.83, P<0.01$)。因此,综合考虑提取效率及与植物硒的相关性,0.1 mol/L KH_2PO_4 浸提法是提取弱酸性富硒土壤中生物有效硒的最适方法。

关键词 富硒土壤;生物有效性;硒形态;单一浸提法;连续浸提法

中图分类号 S151.9 **文献标识码** A **文章编号** 1000-2421(2023)06-0087-09

硒(Se)是人体必需的微量营养元素^[1]。农产品是人体硒营养的主要来源,而其可食部位的硒含量受土壤硒含量及其赋存形态等的影响^[2-3]。中国有近51%的耕地面临土壤硒含量较低或不足的问题,39%~61%的人口硒营养水平低下^[4]。同时,我国也存在多个天然富硒区,如湖北恩施和陕西紫阳等地区^[5-6]。但受土壤硒有效性变化较大的影响,天然富硒区的农产品多存在硒含量变幅大、不稳定等问题。因此,筛选最适的土壤硒生物有效性评价方法尤为重要。

研究表明,土壤总硒作为评估土壤硒含量的指标,可在一定程度反映土壤硒的有效性,但评估效果有限^[7-8]。土壤有效硒才是决定其生物有效性的关键参数^[9-10]。硒在土壤中的有效性受到pH、Eh、有机质、铁氧化物等因素的影响^[11-13]。根据硒与土壤组分的结合方式,可通过连续浸提法将硒分为水溶态、可交换态、铁锰氧化物结合态、有机结合态和残渣态5种形态。其中水溶态硒和交换态硒是土壤中最活跃的硒组分,常被看作植物可利用态(即有效态)硒^[5]。

单一浸提法常用来提取土壤有效硒。浸提剂可分为水浸提剂、中性盐浸提剂、有机络合剂、其他酸性或碱性溶液等,主要有去离子水、 K_2SO_4 溶液、 KCl 溶液、 NaHCO_3 溶液、 KH_2PO_4 溶液、 HCl 溶液、 NaOH 溶液、 DTPA 溶液和 EDTA 溶液等。水常用于水溶态硒的提取,主要依靠水的溶解作用提取水溶性硒,但其提取能力相对较弱^[14]。 K_2SO_4 和 KCl 等中性盐溶液可通过水的溶解和离子交换作用浸提有效硒,但是也存在提取率较低的问题^[15-16]。 KH_2PO_4 溶液和 NaHCO_3 溶液常被用于酸性土壤和石灰性土壤有效硒的提取; KH_2PO_4 溶液的浸提机制为与硒的含氧阴离子发生配位交换作用^[6];而 NaHCO_3 溶液主要通过铁、铝、钙等形成沉淀从而释放吸附态的硒^[17],且多数研究表明这2种浸提剂与植物硒含量的相关性较好^[15, 17]。此外, DTPA 溶液和 EDTA 溶液可通过与吸附硒的金属离子发生配位作用提取硒,常用于石灰性土壤中铁锰氧化物结合态和有机结合态硒的提取^[18]。可见,不同浸提剂的提取机制不同,且提取量及其与植物硒含量的相关性差异较大,需针对

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恩施天然富硒土壤进行有效硒提取方法的研究。

本研究以恩施天然富硒土壤为研究对象,采用连续浸提法和常见的10种单一浸提法对土壤硒形态及有效硒进行提取、测定,并通过分析水稻对不同富硒土壤样品中硒的吸收,结合连续浸提法及单一浸提法测得的土壤有效硒含量与植物硒含量的相关性分析结果,筛选出结果准确、操作简便的有效硒的提取方法,为准确评价富硒土壤中硒的生物有效性及富硒土壤资源的利用提供科学依据。

1 材料与方法

1.1 供试土壤

土壤样品采集于湖北省恩施土家族苗族自治州建始县。共计采集40份耕层(0~20 cm)土壤样品,采样点位置信息见图1。采样时避免田边、路边、沟边和特殊地形位置,每个采集点的土壤样品深浅和样品量保持一致。将土壤样品带回实验室后,置于室内通风阴干,拣去大的植物残体和石块后研磨过孔径0.85 mm筛,存储备用。表1为供试土壤的主要理化性质。

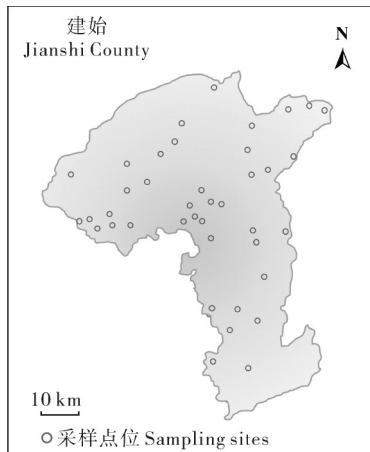


图1 采样点分布

Fig. 1 Soil sampling points in Jianshi County of Enshi

1.2 盆栽试验

为探究不同硒生物有效性的土壤对水稻硒吸收的影响,在华中农业大学玻璃温室中进行了水稻盆栽试验。具体操作如下:将0.5 kg风干土壤装入塑料盆中,以尿素、过磷酸钙和 K_2SO_4 作为基肥,分别以N 0.2 g/kg、 P_2O_5 0.15 g/kg和 K_2O 0.2 g/kg的用量施入土壤。水稻品种为“闽丰优3301”,将水稻幼苗移栽到塑料盆中,每个处理重复3次。在种植期间保持2 cm的上覆水高度,移栽后30 d收获水稻地上部

表1 供试土壤的主要理化性质

Table 1 Related properties of the tested soils

样品编号 Sample No.	pH	有机质含量/ (g/kg) Content of organic matter	总铁含量/ (g/kg) Content of total Fe	总铝含量/ (g/kg) Content of total Al	总锰含量/ (g/kg) Content of total Mn
1	5.32	44.66	35.99	57.50	1.23
2	6.78	79.66	37.38	53.26	0.87
3	5.21	36.33	26.33	42.98	0.79
4	5.72	25.56	34.15	58.86	1.08
5	7.46	21.43	31.05	53.44	1.05
6	6.32	28.34	40.02	63.17	1.72
7	6.53	54.51	40.55	69.27	0.78
8	5.71	30.25	23.52	50.86	0.19
9	6.04	32.02	23.15	40.25	0.57
10	5.78	15.82	30.75	53.02	0.74
11	7.72	34.33	27.11	48.07	0.76
12	6.52	26.63	28.68	56.28	0.22
13	6.65	27.19	28.38	47.37	1.08
14	7.84	43.31	28.00	49.43	1.08
15	6.84	38.7	32.17	48.70	0.68
16	5.84	16.34	27.64	49.49	0.98
17	6.15	32.35	24.05	39.21	0.82
18	6.16	18.96	33.50	64.58	0.63
19	6.32	29.32	29.13	44.55	1.15
20	6.18	32.42	36.95	58.21	1.58
21	6.54	26.79	38.81	60.46	0.97
22	6.91	28.40	31.60	55.22	0.91
23	6.96	64.15	36.33	53.93	0.98
24	5.36	22.39	30.85	50.78	1.04
25	6.06	16.55	31.99	52.23	0.83
26	5.11	15.54	20.18	38.18	0.36
27	5.65	28.05	26.76	46.28	0.82
28	5.93	39.28	31.90	53.19	1.61
29	6.28	20.99	28.26	47.47	1.29
30	7.78	36.84	36.68	64.75	0.67
31	5.50	26.89	29.79	46.77	0.90
32	6.23	35.40	33.12	59.83	0.42
33	5.23	33.40	24.88	41.06	0.72
34	5.4	20.08	35.08	64.24	0.62
35	5.03	29.77	25.92	46.74	0.72
36	7.16	36.73	32.20	55.50	0.30
37	6.67	24.99	27.39	46.98	0.95
38	7.43	67.35	33.04	56.93	0.65
39	4.9	14.30	23.60	37.62	0.37
40	6.17	33.58	31.08	55.57	0.49

分,用去离子水仔细清洗、于60℃烘干至恒质量,然后研磨过孔径0.15 mm筛,储存待测。

1.3 土壤和植物硒含量测定

植物样品用 $HNO_3 - HClO_4(4:1, V/V)$ 消解,在

消化过程中温度保持在180℃。消煮至消化液清亮并冒白烟,取出、冷却后加入6 mol/L的HCl,再次加热至溶液变为清亮并伴有白烟出现时取出,随后定容并过滤。硒含量测定采用原子荧光光谱法(HG-AFS-8220),仪器检测限为0.01 μg/L。土壤总硒的测定除了用HNO₃-HClO₄(3:2, V/V)代替HNO₃-HClO₄(4:1, V/V)之外,其他测定步骤同植物硒含量的测定。同时以小麦籽粒标准样品(GBW-10011, GSB-2)和土壤标准样品(GBW-07404, GSS-4)作为质控样品,硒回收率分别为96.8%±1.7%和

97.3%±1.6%。

1.4 土壤有效硒的测定

分别采用超纯水、0.25 mol/L KCl溶液、0.2 mol/L K₂SO₄溶液、0.5 mol/L NaHCO₃溶液、0.1 mol/L KH₂PO₄溶液、DTPA溶液、0.05 mol/L EDTA溶液、0.1 mol/L HCl溶液和0.4 mol/L HNO₃溶液作为提取剂对供试土壤有效硒含量进行提取、测定^[3, 14-17, 19-20]。其中,使用超纯水提取土壤有效硒的方法与连续浸提法中测定水溶态硒的方法一致,因此,以水溶态硒的测定结果代替(表2)。

表2 不同土壤有效硒浸提方法的测定条件

Table 2 Test conditions of different extraction methods for soil available Se extraction

提取剂 Extractants	浓度/(mol/L) Concentration	土液比(m:V) Soil:Solution	提取方法 Extraction methods
超纯水 Ultrapure water		1:10	25℃, 200 r/min 振荡 2 h
KCl	0.25	1:10	25℃, 200 r/min 振荡 2 h
K ₂ SO ₄	0.2	1:10	25℃, 200 r/min 振荡 2 h
NaHCO ₃	0.5	1:5	25℃, 200 r/min 振荡 2 h
NaHCO ₃	0.5	1:10	25℃, 200 r/min 振荡 2 h
KH ₂ PO ₄	0.1	1:10	25℃, 200 r/min 振荡 2 h
DTPA	/	1:10	25℃, 200 r/min 振荡 1 h
EDTA	0.05	1:10	25℃, 200 r/min 振荡 2 h
HCl	0.1	1:10	25℃, 200 r/min 振荡 1.5 h
HNO ₃	0.4	1:10	25℃, 200 r/min 振荡 1.5 h

注:DTPA浸提剂由0.005 mol/L的二乙烯三胺五乙酸、0.01 mol/L的CaCl₂和0.1 mol/L的三乙醇胺组成。Note:DTPA was composed of 0.005 mol/L DTPA, 0.01 mol/L CaCl₂, and 0.1 mol/L TEA.

1.5 土壤硒形态的测定

土壤硒形态测定采用经过优化的传统连续浸提法^[21]。称取2.00 g过孔径0.149 mm筛的土壤样品放入50 mL聚丙烯离心管。具体操作步骤如下:(1)水溶态硒(soluble Se, SOL-Se),在离心管中加入20 mL去离子水,然后在25℃下以200 r/min振荡1 h。将混合物于4 000 r/min离心10 min,然后过0.45 μm滤膜,收集滤液用以分析水溶态硒的含量。后续每个提取步骤后均进行相同的离心、过滤操作。(2)交换态硒(exchangeable Se, EXC-Se),在步骤(1)的残渣中加入20 mL 0.1 mol/L的KH₂PO₄-K₂HPO₄(pH7.0)溶液,然后在25℃下200 r/min振荡2 h。(3)铁锰氧化物结合态硒(Fe-Mn oxide-bound Se, FMO-Se),在步骤(2)的残渣中加入20 mL 3 mol/L HCl溶液,在90℃水浴下间歇振荡50 min。(4)有机结合态(organic matter-bound Se, OM-Se),在步骤(3)的残渣中加入20 mL 0.1 mol/L的K₂S₂O₈溶液,在90℃水浴下间歇振荡2 h。(5)残渣态硒(residual Se, RES-Se),将最终的土壤残渣置于烘箱中干燥并研磨过孔径0.149 mm筛备用。残渣态硒的测定方法同

土壤总硒测定方法。

1.6 数据分析

试验数据采用SPSS 20.0(IBM Corp, USA)进行处理,采用Origin 2018(OriginLab Corp, USA)和PowerPoint 2016进行图表制作。所列数据为3个重复的均值,采用Pearson相关性分析进行不同形态硒、有效硒与植物硒含量的关系。

2 结果与分析

2.1 土壤硒形态

土壤总硒含量和硒形态测定结果(图2)显示,供试土壤硒含量范围在0.12~3.94 mg/kg,平均值为0.80 mg/kg,55%的土壤样品硒含量超过了富硒土壤标准(0.4 mg/kg),35%的土壤硒含量达到中硒水平(0.175~0.4 mg/kg)。土壤水溶态、可交换态分别为0.12~46.0(均值6.49)、0.58~197.35 μg/kg(均值为37.63 μg/kg);铁锰氧化物结合态、有机结合态及残渣态硒含量分别0.01~0.34(均值为0.08)、0.05~0.88(均值为0.24)、0.04~2.72 mg/kg(均值为0.43 mg/kg)。

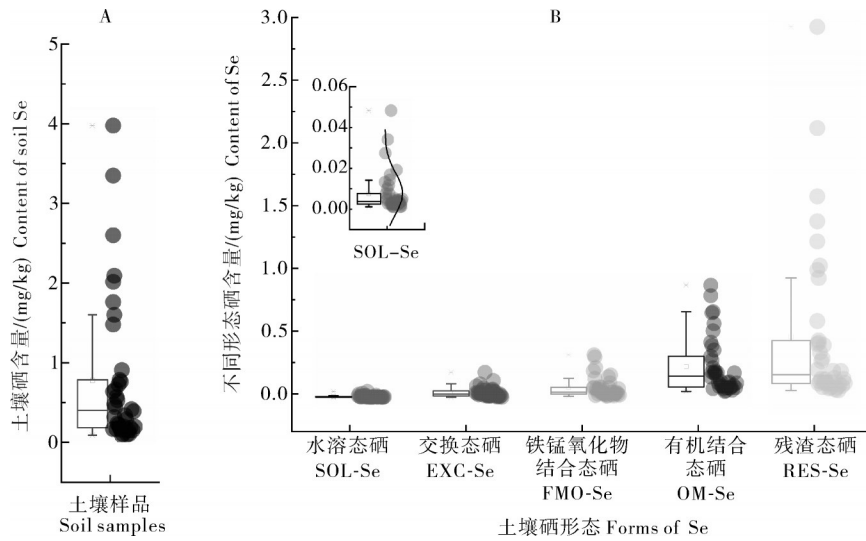


图2 土壤样品总硒含量(A)和硒形态分布特征(B)

Fig. 2 Total concentration(A) and fractions distribution(B) of Se in natural seleniferous soil

2.2 不同浸提方法测定的硒含量

本研究分别采用10种常用方法对土壤有效硒进行了提取、测定。图3为不同浸提方法测定的土壤有效硒的含量。0.5 mol/L NaHCO₃(1:5, m: V)、0.5 mol/L NaHCO₃(1:10, m: V)、0.1 mol/L KH₂PO₄和0.4 mol/L的HNO₃溶液的提取量相对较高,提取量分别为0~253.57 μg/kg(均值为68.41 μg/kg,提取比例为8.59%)、1.69~419.82 μg/kg(均值为110.42 μg/kg,提取比例为13.87%)、0.38~221.30 μg/kg(均值为51.20 μg/kg,提取比例为6.43%)、0.64~451.33 μg/kg(均值为44.56 μg/kg,提取比例为5.60%)。0.25 mol/L KCl、0.2 mol/L K₂SO₄和0.1 mol/L HCl

溶液的提取量低于以上几种单一提取剂,提取的硒含量分别为1.81~48.49 μg/kg(均值为12.87 μg/kg,提取比例为1.62%)、1.04~71.47 μg/kg(均值为10.48 μg/kg,提取比例为1.32%)、0~40.28 μg/kg(均值为12.26 μg/kg,提取比例为1.54%)。0.05 mol/L EDTA溶液的提取量最低,提取比例仅为0.29%。此外,在DTPA提取液中的硒含量也相对较低。

2.3 不同方法提取的土壤有效硒与植物硒含量的关系

分别将土壤总硒、传统的连续浸提法测定的5种土壤硒形态和10种单一浸提剂提取的土壤有效硒与

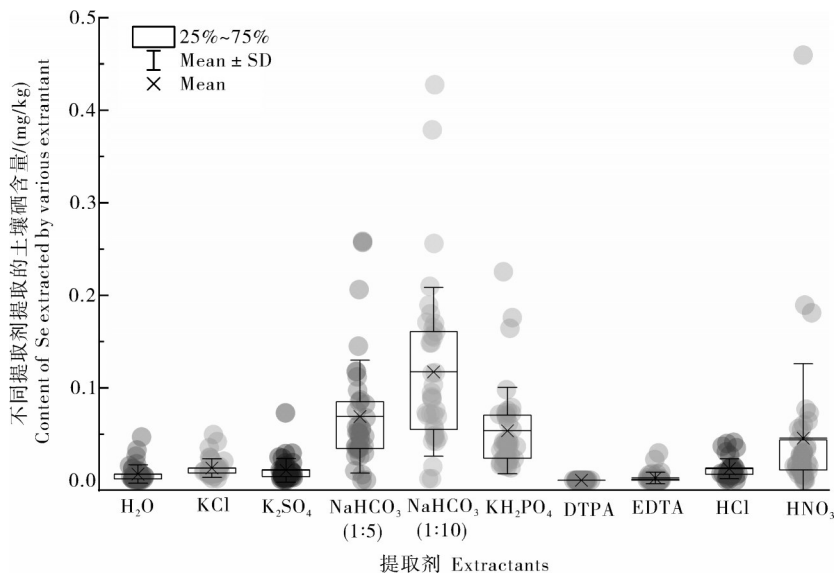
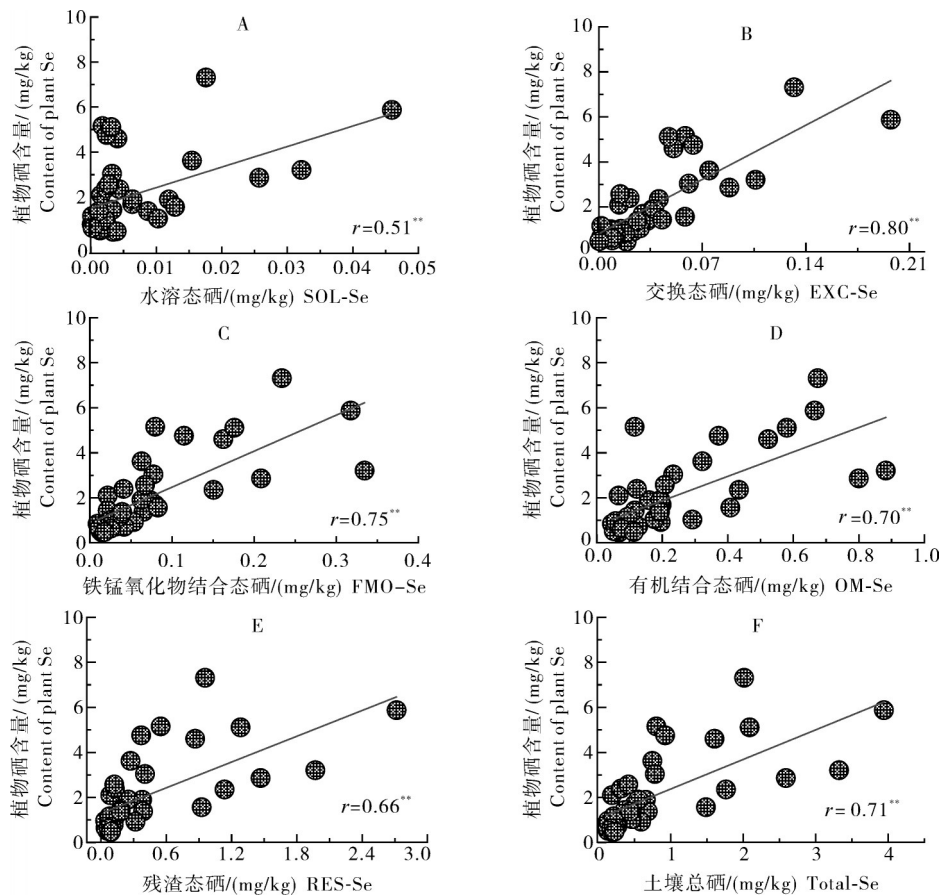


图3 不同浸提方法测定的土壤有效硒含量

Fig. 3 Content of soil available Se measured by various single extraction method

植物硒含量进行相关性分析(图4),结果显示,土壤总硒与植物硒含量之间的相关系数为0.71($P < 0.01$)。不同形态硒与植物硒含量间的相关性由高到低分别为:交换态硒($r=0.80, P < 0.01$)>铁锰氧化物结合态硒($r=0.75, P < 0.01$)>有机结合态硒($r=0.70, P < 0.01$)>残渣态硒($r=0.66, P < 0.01$)>

水溶态硒($r=0.51, P < 0.01$)。土壤中水溶态硒和交换态硒是有效性较高的2种硒形态,在本研究中,植物硒含量与土壤中交换态硒含量的相关系数最高,为0.80($P < 0.01$),与水溶态硒的相关性则相对较低,相关系数仅为0.51,这可能与土壤中较低的水溶态硒含量有关。



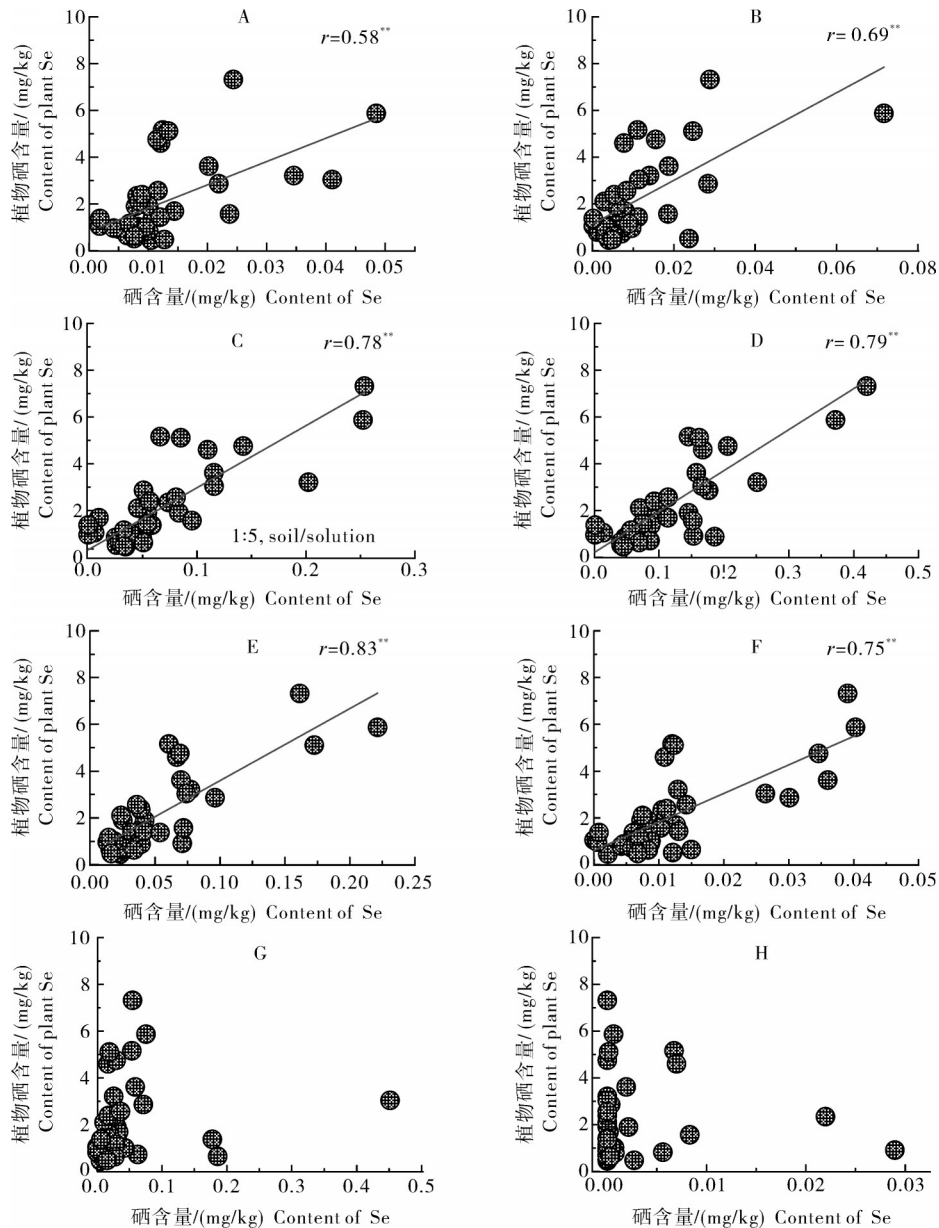
A:水溶态硒 SOL-Se; B:交换态硒 EXC-Se; C:铁锰氧化物结合态硒 FMO-Se; D:有机结合态硒 OM-Se; E:残渣态硒 RES-Se; F:土壤总硒 Total Se; r 代表Pearson相关系数,**代表在0.01的水平上达到显著差异。下同。 r values mean Pearson's correlation coefficients, ** indicates significant difference at 0.01 level. The same as follows.

图4 土壤中不同形态硒与植物硒含量的相关性分析($n=40$)

Fig. 4 Correlation analyses between various soil Se fractions and Se in plants ($n=40$)

在本研究中,采用不同浸提剂提取的土壤有效硒与植物硒含量之间的相关性差异较大(图5)。不同方法提取的土壤有效硒与植物硒含量之间的相关性由高到低分别为:0.1 mol/L KH_2PO_4 ($r=0.83, P < 0.01$)>0.5 mol/L NaHCO_3 (1:10, 土水比) ($r=0.79, P < 0.01$)>0.5 mol/L NaHCO_3 (1:5, 土水比) ($r=0.78, P < 0.01$)>0.1 mol/L HCl ($r=0.75, P < 0.01$)>0.2 mol/L K_2SO_4 ($r=0.69, P < 0.01$)>0.25 mol/L KCl ($r=0.58, P < 0.01$)。其中,0.1 mol/L KH_2PO_4 提取的土壤有效硒与植物硒含量

的相关性最高,而0.25 mol/L的KCl溶液提取的土壤有效硒含量与植物硒的相关性最低。0.1 mol/L的HCl提取的土壤硒含量与植物硒含量之间的相关性也较高($r=0.75, P < 0.01$)。0.25 mol/L的KCl溶液和0.2 mol/L的 K_2SO_4 溶液提取的土壤有效硒占总硒的比例同样较低(分别为1.62%和1.32%),且与植物硒含量之间的相关系数也低于其他几种浸提剂。0.4 mol/L的 HNO_3 溶液和0.05 mol/L的EDTA溶液提取的土壤有效硒与植物硒含量之间不存在显著相关关系。



A: 0.25 mol/L KCl (1:10, soil: solution); B: 0.2 mol/L K_2SO_4 (1:10, soil: solution); C: 0.5 mol/L $NaHCO_3$ (1:5, soil: solution); D: 0.5 mol/L $NaHCO_3$ (1:10, soil: solution); E: 0.1 mol/L KH_2PO_4 (1:10, soil: solution); F: 0.1 mol/L HCl (1:10, soil: solution); G: 0.4 mol/L HNO_3 (1:10, soil: solution); H: 0.05 mol/L EDTA (1:10, soil: solution).

图5 不同浸提方法提取的有效硒含量与植物硒的关系 ($n=40$)

Fig. 5 Relationships between Se extracted by various extractants and Se in plants ($n=40$)

3 讨论

我国的富硒土壤硒含量标准为 ≥ 0.4 mg/kg,本研究中的土壤硒含量均值为0.80 mg/kg,55%的土壤样品硒含量超过了富硒土壤标准,35%的土壤硒含量达到中硒水平(0.175~0.4 mg/kg)。总之,建始县的土壤硒资源分布较为丰富。水溶态硒和交换态硒被认为是生物有效性较高的硒形态,然而本研究中土壤中水溶态和交换态硒的含量较低,硒主要以

有机结合态和残渣态等难以被植物利用的形态存在,这与前人对紫阳富硒土壤的研究结果类似^[6]。较低的水溶态和交换态硒含量可能是造成富硒土壤中硒的生物有效性较低的重要原因。

不同浸提方法提取的土壤有效硒含量差异很大,这主要受到浸提剂的性质和其提取机制的影响。DTPA溶液属于碱性浸提剂,可提取土壤中的水溶态和部分腐殖酸结合态的硒,还能够溶解部分含硒化合物,因此,DTPA溶液对土壤有效硒的提取能力

较强^[16, 22]。EDTA溶液作为螯合剂,在土壤中具有较强的络合能力,可与土壤中的钙镁离子等置换出吸附态的硒,因此,土壤有效硒的提取能力也较强^[16]。然而本研究中EDTA提取液中的硒含量极低,提取率仅为0.29%,在DTPA提取液中甚至没有硒被检测出。推测是由于DTPA和EDTA溶液常用于碱性土壤中硒的提取,而本研究中的供试土壤多为酸性,这可能是提取量较低的重要原因。NaHCO₃溶液属于碱性浸提剂,它产生的碳酸根离子可以与铁、铝、钙等金属离子形成沉淀,从而释放出吸附态的硒酸盐和亚硒酸盐,因而NaHCO₃溶液提取的硒含量最高,且随着水土比的增大提取量也随之变大^[16, 19-20]。KH₂PO₄溶液中的磷酸根离子可与硒竞争土壤固相表面的吸附位点,可将交换态硒从土壤固相释放出来,还可以提取出部分与土壤结合的有机硒,因此,KH₂PO₄溶液提取的硒的含量也较高^[15, 17, 23]。0.4 mol/L HNO₃主要通过酸溶作用提取土壤中的硒,对土壤有效硒的提取能力相对较弱^[19]。KCl和K₂SO₄溶液主要提取的是土壤中的水溶态硒,而土壤中水溶态硒的含量极低,因此,这2种浸提剂提取效果相对较差。与0.4 mol/L HNO₃的提取相似,0.1 mol/L HCl主要通过酸溶作用提取硒,因其浓度较低,对土壤有效硒的提取能力相对较弱^[19]。

不同形态的硒和不同提取方法提取的土壤有效硒与植物硒含量的相关性差异较大。Peng等^[24]发现在外源施加硒酸盐的土壤中,小麦、花椰菜和甘蓝的硒含量与水溶态硒的相关性最高。这是由于在施加外源硒酸盐的土壤中水溶态硒含量较高,而在天然富硒土壤中水溶态硒含量较低^[6, 13, 25-27],交换态硒是天然富硒土壤中植物吸收利用的主要硒形态,因此,本研究中交换态硒与植物的相关性较高,而水溶态硒与植物硒的相关性较低。此外,土壤中铁锰氧化物结合态硒、有机结合态硒和残渣态硒的移动性和生物有效性相对较低^[3],这几种形态硒相对难以被植物吸收利用^[28-29],与植物的相关性较低。0.1 mol/L的HCl主要通过酸溶作用提取有效硒,铁锰氧化物结合态硒是土壤中的重要潜在硒源,将随铁氧化物的溶解而被释放出来,因此,表现出与植物硒显著的相关性。但是其提取量相对较低(仅占土壤总硒的1.54%),不适合作为土壤有效硒的浸提剂。宋晓珂等^[30]发现采用NaHCO₃溶液和KH₂PO₄溶液作为富硒土壤有效硒的浸提剂时,土壤有效硒法的提取量与植物硒含量之间的相关性最高。温国灿等^[31]的研

究也认为KH₂PO₄溶液是酸性土壤和碱性土壤有效硒的最佳浸提剂。此外,在本研究中,NaHCO₃溶液作为浸提剂对土壤有效硒的提取效果也较好,但NaHCO₃溶液作为碱性浸提剂对土壤pH的干扰较大。同时,本研究中的土壤多为弱酸性土壤,NaHCO₃溶液提取的硒与植物硒的相关性相对弱于KH₂PO₄溶液。0.4 mol/L的HNO₃溶液和0.05 mol/L的EDTA溶液提取的土壤有效硒与植物硒含量之间不存在显著相关关系,因此DTPA和EDTA溶液不适用于土壤有效硒的提取。综合以上结果,0.1 mol/L的KH₂PO₄溶液最适用于作为酸性富硒土壤有效硒的化学提取剂。

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Extraction methods of selenium bioavailability in seleniferous soil

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Abstract Most agricultural products produced in naturally seleniferous areas have problems including large variation and instability of Se content. Therefore, it is of great significance to establish a suitable extraction method for the development and utilization of seleniferous soil resources. In this article, 40 soil samples were collected from the naturally seleniferous areas in Jianshi County, Enshi Tujia and Miao Autonomous Prefecture, Hubei Province and used for rice pot experiment to establish a method for extracting effective selenium from acidic and seleniferous soil. The sequential extraction method and 10 commonly used single step extraction methods were used to extract and determine the forms of selenium and available selenium in soil. The correlation between the content of effective selenium extracted by different extraction methods and the selenium absorbed by rice plants was analyzed. The results showed that the content of water-soluble and exchangeable selenium in the acidic and seleniferous soil in Jianshi was relatively low, ranging from 0.12 to 46.0 $\mu\text{g}/\text{kg}$ and from 0.58 to 197.35 $\mu\text{g}/\text{kg}$, respectively. Selenium mainly existed in forms of organic bound and residual selenium that are difficult for plants to utilize. The content of selenium in iron manganese oxide bound state, organic bound state, and residual state was 0.01-0.34 mg/kg , 0.05-0.88 mg/kg , and 0.04-2.72 mg/kg , respectively. Among dozens of single step extractants, 0.5 mol/L NaHCO_3 and 0.1 mol/L KH_2PO_4 solutions had higher extraction efficiency for available selenium in soil. Among different forms of selenium in soil, the correlation between exchangeable selenium and the content of selenium in plant was the highest ($r=0.80$, $P<0.01$), while the correlation between other forms of selenium and the content of selenium in plant was lower than that of exchangeable selenium. Among the single step extraction methods, the content of available selenium in soil extracted with 0.1 mol/L KH_2PO_4 solution and 0.5 mol/L NaHCO_3 solution was higher, but the correlation between the former and the content of selenium in plant was more significant ($r=0.83$, $P<0.01$). It is indicated that the extraction method with 0.1 mol/L KH_2PO_4 is the most suitable method for extracting biologically available selenium from weakly acidic and seleniferous soil if considering the extraction efficiency and its correlation with the content of selenium in plant.

Keywords seleniferous soil; bioavailability; forms of selenium; single-step extraction method; sequential extraction methods

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