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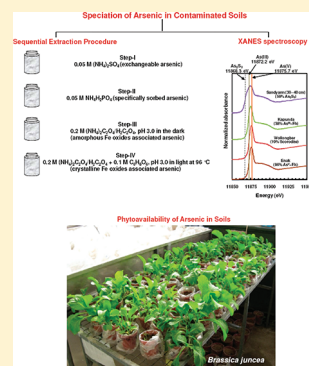
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S Supporting Information

ABSTRACT: In this study, a sequential extraction procedure (SEP) and X-ray absorption near edge structure (XANES) spectroscopy were used to determine the solid-phase speciation and phytoavailability of arsenic (As) of historically contaminated soils from As containing pesticides and herbicides and soils spiked with As in the laboratory. *Brassica juncea* was grown in the contaminated soils to measure plant available As in a glasshouse experiment. Arsenic associated with amorphous Fe oxides was found to be the dominant phase using both SEP and XANES spectroscopy. Arsenic predominantly existed in arsenate (As^{V}) form in the soils; in a few samples As was also present in arsenite (As^{III}) form or in scorodite mineral. Arsenic concentration in shoots showed significant ($p < 0.001\text{--}0.05$) correlations with the exchangeable As ($r = 0.85$), and amorphous Fe oxides associated As evaluated by the SEP ($r = 0.67$), and As associated with amorphous Fe oxides as determined by XANES spectroscopy ($r = 0.51$). The results show that As in both fractions was readily available for plant uptake and may pose a potential risk to the environment. The combination of SEP and XANES spectroscopy allowed us the quantitative speciation of As in the contaminated soils and the identification of valence and mineral forms of As. Such detailed knowledge on As speciation and availability is vital for management and rehabilitation of As-contaminated soils.



INTRODUCTION

Arsenic contamination of soil is a major environmental concern due to the toxic and carcinogenic nature of some As compounds.^{1,2} Agriculture use of As-based pesticides and herbicides, ore mining and smelting, and CCA treatment of wood and pesticides manufacturing processes are the major anthropogenic sources of soil As contamination.^{1,2} Arsenicals (as sodium arsenite) were applied to control the southward migration of cattle tick across New South Wales (NSW) in Australia from the early 1900s to 1955. Over 1500 dips were constructed along the eastern coast of Australia with many of these being located in northern NSW.² Arsenic-containing herbicides were also used to suppress weed growth along the former railway corridors in South Australia over 30 years ago.³ The repeated application of As-based pesticides and herbicides led to As concentrations of up to 3000 and 1400 mg kg⁻¹ in soils at the cattle dip sites and railway corridors, respectively.^{2,3} Arsenic in the soils adjoining these sites significantly exceeds the ecological investigation level (EIL, 20 mg kg⁻¹) in the soil in Australia.⁴ Limited research suggests that As exists in more soluble and toxic forms at these sites and thus presents significant environmental and health risks.^{2,5} The site-specific evaluation of As-contaminated soil is, therefore, imperative to determine the chemical forms of As and to evaluate phytoavailability of As. Such information would be important for risk assessment and for the development and implementation of suitable management and remediation strategies.

Sequential extraction procedures have been previously used to delineate chemical forms of As in soils such as nonspecifically and specifically sorbed As, As occluded in amorphous and crystalline Fe oxides, and recalcitrant As.^{5–8} In a previous study, McLaren et al.⁵ used a sequential fractionation method for As partitioning in contaminated soils. They reported that As was mostly adsorbed onto amorphous Fe oxides (0.1 M NaOH extractable) in soils sampled from disused cattle dip sites, and a substantial proportion of As was highly labile (0.05 M NaHCO₃ extractable). The sequential extraction procedures are reported to have some limitations which include the partial dissolution of a required fraction,⁹ dissolution of the nontarget phase, and a partial recovery of the desired phase due to readsorption or reprecipitation reactions.^{8,10,11} The redox forms of As in soils are also not detected using a sequential extraction procedure.^{7,8}

X-ray absorption fine structure (XAFS) spectroscopy is a powerful technique, which has been used to determine the oxidation state and chemically bound forms of As in contaminated soils.^{12–15} Cances et al.¹³ observed that As predominantly occurred as As^{V} , and approximately 80% of the As was bound to the amorphous Fe oxides throughout the soil profile at a former arsenical processing plant. Arai et al.¹² investigated an

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As-contaminated site close to a pesticide (PbHAsO_4) manufacturing plant and observed that As in the oxidized (0–10 cm) layer was mainly present as As^{V} in association with the amorphous Fe oxides (71%). Recently, Meunier et al.¹⁴ determined mineralogical composition of As in As-enriched tailings and soils from abandoned gold mine sites using XANES spectroscopy. They also studied the effect of various mineral forms of As on the bioaccessibility of As using an *in vitro* physiologically based extraction test (PBET). The authors found that As was present in mineral forms such as arsenopyrite, scorodite, realgar, and kankite in the studied samples. The bioaccessibility of As was <1% of the total As in arsenopyrite and scorodite and ranged from 2–7% in As-bearing amorphous Fe arsenate and Fe oxyhydroxides minerals.¹⁴

Previous research, however, has not used a combination of SEP and XANES spectroscopy, which could be very powerful in delineating various chemical (labile, sorbed, mineral bound), mineral, and oxidation states of As in contaminated soils. Therefore, this study was conducted employing SEP and XANES spectroscopy to determine As forms. In addition, the phytoavailability of As in the soils was estimated in a glasshouse experiment to indicate the bioavailability of As in terms of plant available As in a range of As-contaminated soils. The primary objectives of this study were to (1) determine the solid-state speciation of As in contaminated soils sampled from historical cattle dip sites and railway corridor and laboratory spiked soils using the combination of a SEP and XANES spectroscopy and (2) evaluate the phytoavailability of As in soils using *Brassica juncea* as a test plant.

MATERIALS AND METHODOLOGY

Soil Sampling. Surface (0–10 cm) samples were collected from 16 As-contaminated cattle dip sites in northern NSW and along 3 railway corridors in South Australia, using a (\varnothing 6 cm) stainless steel soil corer. Subsurface samples at a depth of 30–40 cm were also taken from Blackwoods and Sandyarm sites, hereafter referred to as Blackwoods30–40 cm and Sandyarm30–40 cm. A number of soil cores (8–10) were collected from each of the contaminated sites, which were mixed together to make a composite soil sample (1.5–2 kg) for the site. To obtain a wide range of As-contaminated soils for the study, four soils, Westwood, Lansdowne, Lucerne and Narrabri, with a wide ranging pH, free Fe and clay contents, were spiked with As (Supporting Information (SI) Table S2; Figure S1). Except for the sandy Lansdowne soil, the soils were spiked with three levels of As (100, 200, and 400 mg kg^{-1} As using $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$); the Lansdowne soil was spiked with 10 and 20 mg kg^{-1} As.

Physicochemical Analysis. The soil samples were dried at 40 °C, ground to obtain <2 mm fraction, and homogenized. These samples were used for the glasshouse experiment and for the laboratory and spectroscopic analyses. The soils were characterized for pH in a 1:5 soil:solution (0.01 M CaCl_2) suspension¹⁶ and for cation exchange capacity (CEC) using the silver-thiourea method.¹⁷ The amorphous and free Fe were extracted using ammonium oxalate-oxalic acid¹⁶ and dithionite-citrate bicarbonate (DCB)¹⁸ solutions, respectively (see the SI). The selected physicochemical properties of the soil samples are presented in SI Table S2.

For the total As analysis in soils, subsamples (approximately 15 g) were ground to <200 μm and digested in acids using the methodology described by Huang and Fujii.¹⁹

Phytoavailability Pot Experiment. Small size plastic pots (\varnothing 8 cm; 0.5 kg capacity) internally lined with the polythene bags were filled with the As-contaminated and spiked soils (350 g in each pot). For each soil three replicates were used in the experiment. *Brassica juncea* L. (Indian mustard) was grown in the glasshouse for eight weeks; the temperature in the glasshouse ranged from 16 to 30 °C during the experimental period. After eight weeks, shoots were harvested. The harvested material was washed using a three step washing procedure (tap water–0.1 M HCl–deionized water) as described elsewhere²⁰ and dried at 70 °C for 48 h. The shoot dry matter yield was recorded, and the samples were then ground (<1 mm) and digested in a mixture (1:1) of concentrated nitric and perchloric acids²¹ for As analysis (detail in the SI).

Sequential Extraction Procedure (SEP). A four step slightly modified SEP of Wenzel et al.⁸ was employed to partition As in the soil samples. In the modified SEP, Step-I was repeated twice, and a washing step using 0.05 M NaCl solution was included after each extraction step (SI Table S1). The washing solution was then mixed with the preceding step solution. The washing step was introduced to remove As in the entrained solution after each extraction step and thus minimize overlap and readsorption or precipitation of As between steps. Step-I involved the extraction of exchangeable or readily available As (0.05 M $(\text{NH}_4)_2\text{SO}_4$), and Step-II released specifically sorbed As fraction using phosphate solution (0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$). The third step involved the extraction of As associated with amorphous Fe oxides (0.2 M $(\text{NH}_4)_2\text{C}_2\text{O}_4/\text{H}_2\text{C}_2\text{O}_4$), and Step-IV dissolved As bound to crystalline Fe oxides (0.2 M $(\text{NH}_4)_2\text{C}_2\text{O}_4/\text{H}_2\text{C}_2\text{O}_4$ + 0.1 M $\text{C}_6\text{H}_8\text{O}_6$). The samples were centrifuged at 3000 rpm for 15 min after each extraction step, filtered using a Whatman no. 1 filter paper, and acidified by adding 1 M HCl before As analysis. Arsenic in the residual phase was calculated by subtracting the sum of the four fractions of the SEP from the total As concentration in soil.

Arsenic in soil and plant digests and in soil extracts was determined using a Varian 220Z hydride-generation atomic absorption spectrometer. The QA/QC details are given in the SI.

XANES Spectroscopy. The reference compounds used for the XANES spectroscopy included scorodite ($\text{FeAsO}_4 \cdot 2\text{H}_2\text{O}$), As^{V} adsorbed to goethite (hereafter referred to as $\text{As}^{\text{V}}\text{--Gt}$), $\text{As}^{\text{V/III}}$ adsorbed to ferrihydrite ($\text{As}^{\text{V/III}}\text{--Fh}$),²² As^{V} coprecipitated with ferrihydrite ($\text{As}^{\text{V}}\text{--Cop--Fh}$),²³ prepared following the methods described in detail in the SI. Natural samples of arsenopyrite (FeAsS), orpiment (As_2S_3), and realgar (AsS), obtained from South Australian Museum, were also included as reference compounds. Two scans in the XANES region were collected for each of the reference compound and soil samples. The experimental details for the XANES analysis are given in the SI. XANES spectra for two samples (Boorie and Wollongbar) were also obtained after Step-II and Step-III of the SEP.

The oxidation state of As was found to be associated with a shift in the white line energy position of As^{III} (11,872.2 eV) and As^{V} (11,875.7 eV).²⁴ The software *Average* was used for averaging the raw spectra, while smoothing, normalization, and linear least-squares combination fits (LCF) were performed using *Athena*.^{25,26} LCF of the XANES spectra of reference compounds with those of the soil samples was employed to estimate As forms in the soil (detailed description for the selection of reference compounds for the LCF analysis is given in SI). The goodness of fit and best possible combination of reference compounds describing the fit quality for soil samples were selected based on

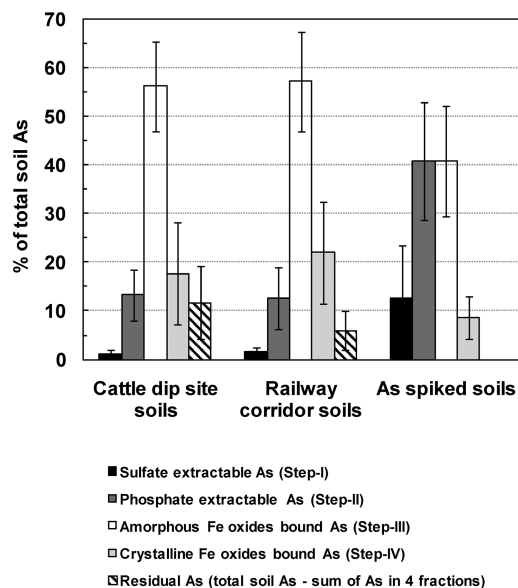


Figure 1. Sequential extraction procedure applied to the cattle dip sites, railway corridor, and As spiked soil samples ($n = 18, 3$, and 11 , respectively). Means of each extracted fraction are presented as percent (%) of the total soil As concentration present in the contaminated and As spiked soils. The residual fraction of As was calculated by subtracting the sum of the four fractions from the total As concentration in each of the soils. The bars represent \pm SD around the mean values.

the R-factor value that is equivalent to the normalized sum of the residuals^{25–27}

Normalized sum of residuals (R-factor)

$$= \sum((y - y_{fit})^2) / \sum(y^2)$$

Data Analysis. The correlations and basic statistical analysis were performed using GenStat version 12.1.²⁸ The amounts of As (mg kg^{-1}) associated with amorphous and crystalline Fe oxides phases in soil were calculated from the LCF predicted proportions of these fractions using XANES spectroscopy.

RESULTS AND DISCUSSION

Soil pH for majority of the samples was <6 except for the Goolmangar (7.02), Murray Bridge (6.93), Adelaide Hills (6.70), and Narrabri (7.53) soils (SI Table S2). Free Fe concentration varied between 0.07 and 15.9 wt % in the soils with <1 wt % in the lighter textured soils i.e., Dungarubba, Blackwoods, Blackwoods30–40 cm, Sandyarm, Sandyarm30–40 cm, Murray Bridge, and Lansdowne samples. Three samples (Faulkners, Dunoon, Wollongbar) contained >5 wt % free Fe, with a notably high Fe concentration (15.9 wt %) in the Wollongbar soil. Amorphous Fe concentration varied between 0.07 and 1.16 wt % in the soil samples (SI Table S2), and it constituted 5.06–97.9% of the free Fe content with a mean value of 40.1%.

Total As in the Soil. Total As concentration in the soils ranged between 10 and 1406 mg kg^{-1} ; for cattle dip sites, As ranged from 21– 1406 mg kg^{-1} whereas for the railway corridor soils from 119– 257 mg kg^{-1} . Dungarubba, Sandyarm, and Sandyarm30–40 cm soils contained relatively low As concentration with values $\leq 34 \text{ mg kg}^{-1}$. Total As concentration increased with depth for the two samples (Sandyarm and Blackwoods),

indicating leaching of As in these sandy soils, which is in agreement with the previous results.⁵

Arsenic Fractionation in Soils Using a SEP. Exchangeable (readily available) As (Step-I) constituted less than 3.5% of the total soil As, except for three As spiked soils, Lansdowne, Narrabri, and Lucerne where it was significantly higher, with values up to 34.7% (SI Table S3). The greater amount of As released during the first extraction step in As spiked soils (see Figure 1) could be related to the soluble form of added As and shorter equilibration time of added As (6 weeks) in the spiked soils.^{2,29,30} Negligible As removal ($<0.3\%$ of the total soil As) in the exchangeable pool occurred in Faulkners, Dunoon, and Wollongbar samples, despite their high total As contents ($>400 \text{ mg kg}^{-1}$), which can be attributed to the presence of high free Fe contents (>5 wt %) in these samples. Conversely, a relatively higher proportion of As (3.1%) was extracted in the Sandyarm sample, which could be due to the lowest free Fe concentration (0.07 wt %) in this sample.

The specifically sorbed As (Step-II) was the second dominant phase, and it constituted 6–66% of the total As. Similar to the exchangeable As phase, the As spiked soils showed a greater proportion of As in this fraction with maximum As extraction in the Narrabri soil (66%) spiked with 100 mg kg^{-1} As (SI Table S3). The substantial release of As in Step-II indicated that As can be potentially mobilized as a result of phosphate application to these soils.^{2,31} However, further studies are warranted to determine the effect of phosphate on the mobility and bioavailability of As to plants under field conditions. For all samples, As was primarily associated with amorphous Fe oxides (Step-III), and it ranged from 22–75% with a mean of 51% of the total As. Relatively, lower values were obtained in the Step-III for the As spiked soils, as a higher proportion of As was extracted (mean = 54%) in the first two steps of the SEP (Figure 1; SI Table S3).

Arsenic recovered upon the dissolution of crystalline Fe oxides in the Step-IV constituted $\leq 24\%$ of the total soil As, except for the Faulkners, Dunoon, Wollongbar, and Adelaide Hills samples where it was $\geq 34\%$. The crystalline Fe content in these four samples was $\geq 87.5\%$ of the free Fe content; therefore, it could be expected that As was adsorbed or coprecipitated in greater proportion with the crystalline Fe oxides in these soils (SI Table S3). The residual phase of As was $\leq 11.7\%$ of the total As in the cattle dip site and railway corridor soils, while no residual As was found in the As spiked soils (Figure 1). A low amount of residual As in historically contaminated soils reflects that these soils could potentially release As in the environment due to a greater proportion of As being present in the potentially mobilizable phases.

The differences in As partitioning between the As spiked and long-term contaminated (cattle dip sites and railway corridor) soils (Figure 1, SI Table S3) possibly occurred due to the variation in the equilibration or aging time of the soils.^{30,32} In the spiked soils a much higher proportion of As was removed in the Step-I and Step-II (mean = 12.7 and 41%) of the SEP than that of the long-term contaminated soils (mean = 1.2 and 13%). This could be attributed to the shorter equilibration time of added As as As^{V} in the spiked soils compared to the prolonged aging (over 40 years) in the contaminated soils.³²

Amorphous Fe ($r = 0.63$; $p < 0.001$) and organic carbon ($r = 0.40$; $p < 0.05$) in the contaminated and spiked soils were positively correlated with amorphous Fe oxides bound As (Step-III). The disparities in As speciation among the four artificially As-contaminated soils were related to their varying soil properties,

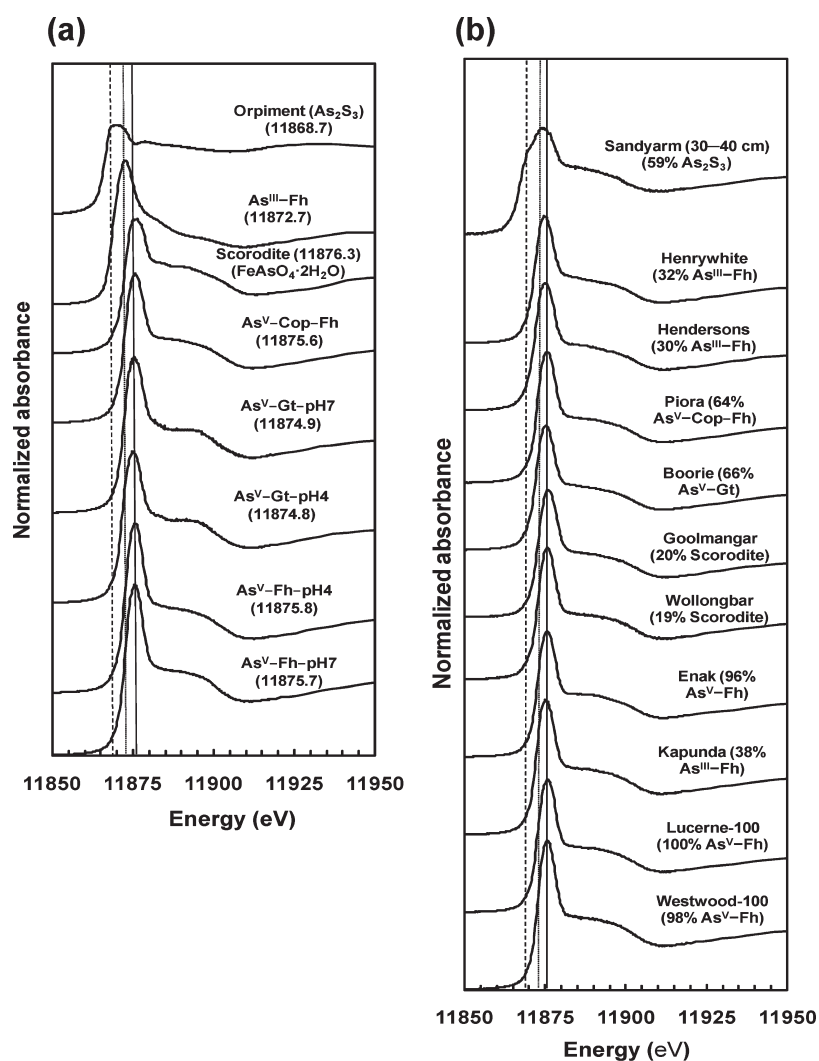


Figure 2. Arsenic K-edge XANES spectra of (a) reference compounds and (b) selected soil samples from the cattle dip sites, railway corridor, and As spiked soils. Solid (—), dotted (•••), and dashed (---) lines drawn at 11875.7, 11872.2, and 11868.7 eV represent the position of white lines for arsenate (As^{V}), arsenite (As^{III}), and orpiment (As_2S_3), respectively. The white line energy values (eV) are given in the parentheses for each of the reference compounds.

such as pH, free Fe, and clay contents. For example, lower As in the exchangeable phase of the Westwood soil ($\leq 2.2\%$) was attributed to the higher free Fe content (3.3%) in this sample. Conversely, higher As removal in the Step-II of Narrabri soil could be due to its relatively lower free Fe content (1.28%), alkaline pH (7.53), and higher clay content (60%).^{2,33} Similarly, Juhasz et al.²⁹ reported that higher Fe content and lower pH in a Red Ferrosol (9.96% Fe, pH 5.8) than that of a Brown Chromosol (0.8% Fe, pH 7.8) reduced As bioavailability up to 75%, after 12 months of incubation. Our results also indicate that soil properties mainly free Fe content and pH appeared to affect the distribution and phytoavailability of As in the spiked soils, albeit all spiked soils were equilibrated for the same time.^{7,8}

Arsenic Speciation Using XANES Spectroscopy. The As K-edge XANES spectra of the reference compounds and selected soil samples are presented in Figure 2a,b. The white line energy position of As (As^{V} and As^{III}) sorbed on Fe oxides and As present in mineral forms was in the following order: scorodite > As^{V} -Fh, As^{V} -Cop-Fh > As^{V} -Gt > As^{III} -Fh > As_2S_3 (Figure 2a). The LCF results from XANES spectra of soil samples showed that As

was predominantly present in the As^{V} form and mainly associated with the amorphous Fe oxides (14–78% of the total As) (Table 1). Arsenic was depicted to present as As^{V} - Fe^{III} coprecipitates in two samples, Piora (64%) and Murray Bridge (28%). Previous studies on As speciation in the As-contaminated mining and pesticides manufacturing sites also showed the predominance of As^{V} associated with amorphous Fe oxides.^{12–14} Arsenic in this fraction is considered to be a potential source of As release to the environment as indicated by Meunier et al.,¹⁴ who investigated As speciation in tailings and mining-impacted soils. They measured bioaccessibility of As in gastric and intestinal phases and reported higher As bioaccessibility ($\sim 10\%$) in the samples having greater proportion of As-bearing amorphous phases (amorphous Fe oxides or calcium-Fe-arsenate) than those where As was present in arsenopyrite and scorodite ($< 1\%$) minerals. Our XANES and SEP (Step-III) data also showed the greatest proportion (51% and 75% of total As) of As being present in the potentially available (As-bearing amorphous Fe oxides) form (Figure 1; Table 1). In the current study, higher proportion of As was found in this phase than those reported by

Table 1. Linear Least-Squares Combination Fits of XANES Spectra of Reference Compounds with Those of Soil Samples (*n* = 30) Analyzed^j

soil samples ^h	reference compounds (% weights) ⁱ				R-factor ^e
	As ^V –Fh ^a	As ^V –Gt ^b	As ^{III} –Fh ^c	Scorodite ^d	
<i>Cattle dip sites (n = 18)</i>					
Dungarubba	57	4	35	4	0.0034
Hendersons	61	6.5	30	2.6	0.0022
Piora	26 (64) ^f	—	10	—	0.0033
Sneesbyslane	31	69	—	—	0.0040
JBM	92	—	8	—	0.0041
Boorie	34	66	—	—	0.0019
Henrywhite	48	14	32	6	0.0031
Enak	96	—	—	4	0.0005
Faulkners	67	—	20	13	0.0041
Dunoon	79	21	—	—	0.0033
Nimbin	78	—	16	6	0.0028
Blackwoods	81	19	—	—	0.0049
Blackwoods30–40 cm	89	11	—	—	0.0023
Alexanders	96	—	—	4	0.0005
Goolmangar	80	—	—	20	0.0016
Sandyarm	61 (17) ^g	—	—	22	0.0066
Sandyarm30–40 cm	14 (59) ^g	3	24	—	0.0083
Wollongbar	81	—	—	19	0.0008
<i>Railway corridor (n = 3)</i>					
Murray bridge	52 (28) ^f	—	20	—	0.0035
Adelaide hills	96	4	—	—	0.0008
Kapunda	56	6	38	—	0.0041
<i>As spiked soils (n = 9)</i>					
Westwood-100	98	—	—	—	0.0006
Westwood-200	97	—	—	—	0.0006
Westwood-400	100	—	—	—	0.0005
Narrabri-100	96	—	4	—	0.0177
Narrabri-200	98	—	2	—	0.0007
Narrabri-400	95	—	—	—	0.0032
Lucerne-100	100	—	—	—	0.0003
Lucerne-200	96	4	—	—	0.0005
Lucerne-400	97	3	—	—	0.0014

^a As^V adsorbed to ferrihydrite. ^b As^V adsorbed to goethite. ^c As^{III} adsorbed to ferrihydrite. ^d Mineral phase of As^V–Fe^{III} (FeAsO₄·2H₂O). ^e Normalized sum of the residuals (R-factor) = $\sum((y - y_{fit})^2) / \sum(y^2)$. ^f As^V coprecipitated ferrihydrite (As^V–Cop–Fh). ^g Orpiment (As₂S₃). ^h All soil samples are collected at 0–10 cm depth, except for the two samples mentioned at 30–40 cm. ⁱ LCF results are presented with <±5% delta weights. ^j Fit results correspond to XANES spectra of selected soil samples plotted in Figure 2.

Meunier et al.¹⁴ (2–7%) during dissolution of As-bearing amorphous forms, which could be attributed to the differences in source of As contamination in the two studies, as mentioned earlier.

LCF results showed that >95% of the total As was present in association with the amorphous Fe oxides in the As spiked soils (Figure 2; Table 1); the Lansdowne samples were excluded from the analysis because of their low As contents. These results are different to the SEP data where upon dissolution of the amorphous Fe oxides (Step-III) 22–57% (mean = 41.7%) of the total As was extracted from the three spiked soils.

The results indicate that XANES spectroscopy may overlook the exchangeable and specifically sorbed fractions of As in historically contaminated and spiked soils. While the SEP allowed us to determine these two highly mobile As phases in the studied soils, notably in the As spiked soils where more than 50% As was extracted in the first two fractions of the SEP. XANES spectra of two soils (Boorie and Wollongbar) was also analyzed after Step-II and Step-III of the SEP to elucidate any change in the spectral features after removal of As in respective steps of the SEP (SI Figure S2). The data for these two soils (after Step-II and Step-III) show nearly identical XANES spectra. The lack of a reference compound representing only the exchangeable fraction of As might be one of the drawbacks in the XANES analysis. The results suggest that the combined use of SEP and XANES is a powerful approach to determine the presence of more labile (and toxic) forms of As in soils which could be underestimated using XANES spectroscopy alone.

Arsenate was also detected to present in adsorbed form onto the surface of crystalline Fe oxides (mean = 19%) (see SI Table S3). In addition to the presence of As^V–Fe oxides adsorption complexes, a mineral phase of As^V, scorodite (FeAsO₄·2H₂O), was also identified in 10 of the 18 samples from cattle dip sites. Scorodite has been previously reported to occur mostly in the As-contaminated mining sites.^{14,15}

Arsenic occurred in As^{III} oxidation state in association with the amorphous Fe oxides in the cattle dip sites and railway corridor samples (Table 1), with the maximum value of 38% observed in Kapunda soil (Figure 2). Arsenite was detected in two of the As spiked soils, where it was <5% of the total As. Reduction of As^V to As^{III} due to the high intensity synchrotron X-rays was discounted considering the low levels of As^{III} observed in the As spiked soils. In a previous study, Smith et al.³⁴ used a chemical method for As speciation in herbicide impacted railway corridor soils and reported up to 40% of the total As in As^{III} form. They postulated that regular application of As^{III}-based herbicides may have led to the increased sorption of As^{III} over As^V on the surface of amorphous Fe oxides under alkaline (pH > 7) soil conditions. This mechanism may explain the presence of As^{III} in the two railway corridor As^{III}-herbicides contaminated soil samples (see Table 1).^{33,34} The presence of As^{III} in the oxidized and acidic soil conditions in the As^{III}-pesticides impacted cattle dip site soils (see Table 1) could possibly be attributed to the microbial reduction of As^V to As^{III} as reported by Turpeinen et al.³⁵ These authors suggested that microbes could transform As^V to As^{III} under aerobic conditions in As-contaminated soils with acidic pH (4.4–4.7). Reduction of As^V can also occur due to complex redox environments that exist in rhizosphere soils. However, it is not immediately evident what factors contributed toward the occurrence of As in As^{III} form only in these soils; further site-specific detailed examinations are warranted to elucidate the factors controlling the oxidation–reduction of As in soils.

A decrease in the white line energy position (11870.03 eV) in the Sandyarm sample collected from 30–40 cm depth (see Figure 2) indicated the presence of orpiment-like precipitates (As₂S₃, 59%), an As^{III}–sulfide mineral phase of As. The presence of As in this form might be due to the presence of sulfur added through superphosphate fertilizers. The presence of microbes and prevailing reducing conditions in the subsurface soil may have led to the formation of As^{III}–S²⁻ species as reported by Arai et al.¹² for a contaminated site near to an arsenical pesticides manufacturing plant.

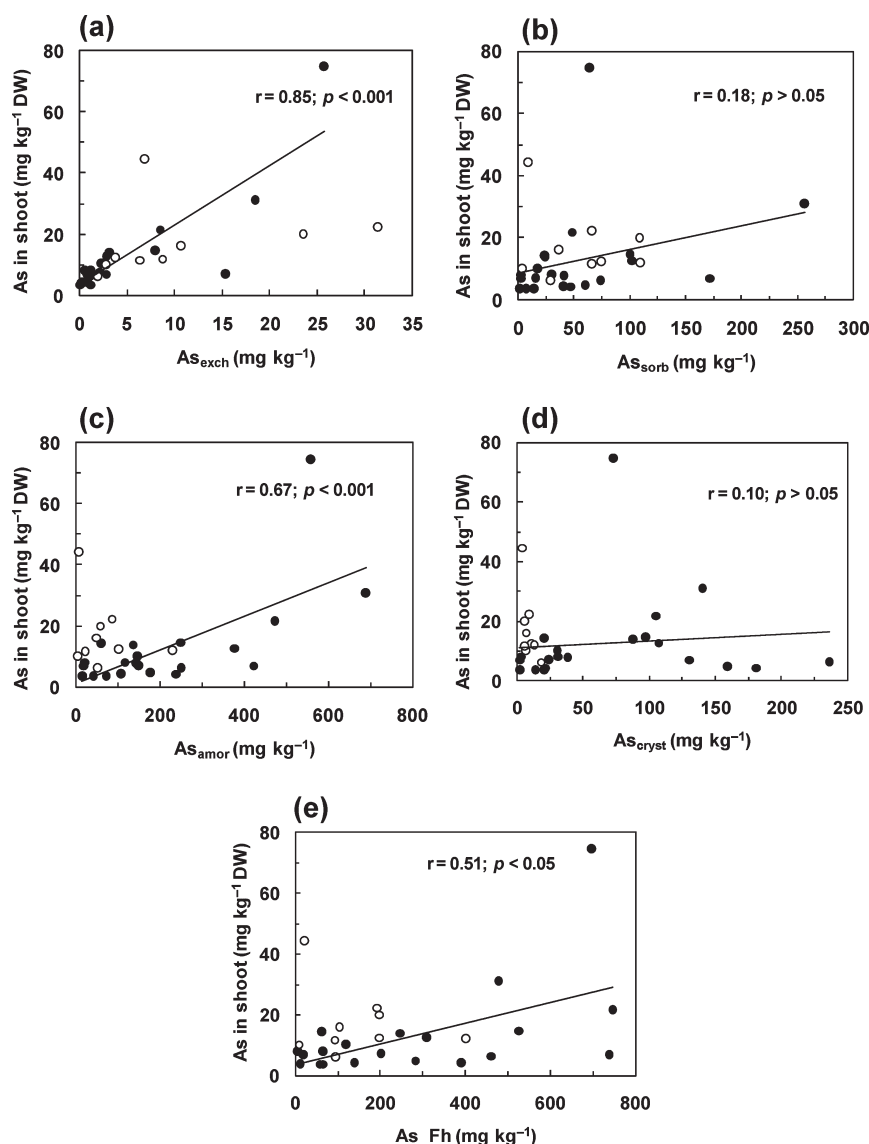


Figure 3. Arsenic concentration in dry shoots of *B. juncea* in relation to the sequentially extracted (a) exchangeable As (Step-I); (b) specifically sorbed As (Step-II); (c) amorphous Fe oxides bound As (Step-III); (d) crystalline Fe oxides bound As (Step-IV); and (e) As determined using XANES associated with amorphous Fe oxides. Cattle dip sites and railway corridor soils (●); As spiked soils (○). The correlations are presented excluding the As spiked soils data due to the considerable differences in aging time.

Arsenic Phytoavailability to *B. juncea* in Relation to SEP and XANES Spectroscopy. Arsenic concentration in the shoot of *B. juncea* ranged from 3.6–74.8 mg kg⁻¹ with the maximum value (74.8 mg kg⁻¹) observed in Blackwoods30–40 cm sample (SI Table S3). The shoot As concentration varied among the studied samples depending upon the degree of contamination and plant availability of As in the soils. The lighter textured (clay ≤ 6%) soils with low free Fe content (≤ 0.22%) including Dungarubba, Sandyarm, and Sandyarm30–40 cm exhibited relatively greater plant As concentration, although the total As concentration in these soils was much lower than the many other contaminated soils (see SI Table S3). The concentration of As in the plant shoot was found to be greater in all samples than the normally reported value of 1.5 μg kg⁻¹ for plants grown in uncontaminated soils.³⁶ The phytotoxicity symptoms of As such as marked reduction in plant growth were also observed across all the contaminated and As spiked soils.^{37,38} Similarly, Jiang and

Singh³⁷ reported a substantial decrease in the dry biomass yield of rye grass and barley plants due to the application of As, which was attributed to the inhibitory effect of As (as As^V) on the phosphate uptake pathway leading to insufficient levels of phosphorylated compounds. Arsenic in As^{III} form is known to have twice as much phytotoxicity as As^V, since it rapidly combines with the dithiol functional groups and destroys the functioning of sulfhydryl enzymes, thereby causing the membrane degradation and immediate cell death. There was greater As toxicity that severely affected plant growth (qualitative observations) and markedly reduced dry matter yield (data not shown) of *B. juncea* plants in Dungarubba, Sandyarm, and Sandyarm30–40 cm soils.^{1,38} This supports our findings that As in these particular soils exists in highly toxic (As^{III}) and plant available form (Table 1).

The shoot As concentration of *B. juncea* in all As spiked samples was comparatively greater than the long-term contaminated soil

samples which agrees with the SEP data showing greater proportion of As in the first two steps (SI Table S3). Among the As spiked samples, maximum shoot As concentration of 44.5 mg kg^{-1} was observed in Lansdowne-20 followed by Lucerne-200 and Narrabri-200 samples (SI Table S3). The plants in Narrabri-400 and Lucerne-400 soils died immediately after germination as a result of severe As toxicity at these high As levels.³⁸ The lower shoot As concentration observed in the Westwood samples ($6.4\text{--}12.4 \text{ mg kg}^{-1}$) compared to the other three As spiked samples could be related to the minimum amount of As in the exchangeable phase in this soil.

The concentration of As in the plant shoot showed a highly significant ($p < 0.001$) positive correlation with the exchangeable As ($r = 0.85$), indicating that As in this phase is readily available to plants (Figure 3a). The results are in agreement with those of Anawar et al.,³⁹ who also reported a significant positive relationship ($r = 0.94$; $p < 0.05$) between exchangeable As and plant As concentration in alfalfa. Arsenic concentration in shoots was also significantly correlated with the amorphous Fe oxides bound As fraction estimated by SEP in Step-III ($r = 0.67$; $p < 0.01$) and XANES ($r = 0.54$; $p < 0.05$) (Figure 3c,e). Our data indicate that As associated with amorphous Fe oxides was also plant available. A significant positive correlation was observed between the amorphous Fe oxide content and As associated with amorphous Fe oxides fraction estimated using SEP (Step-III, $r = 0.63$; $p < 0.01$) and XANES ($r = 0.50$; $p < 0.01$) (SI Figure S3), which further supports the earlier suggestion. The phytoavailability of As from this fraction could possibly be attributed to the release of As from amorphous Fe oxides phase, since this As fraction is considered less stable than those bound with the crystalline forms.^{40,41} Arsenic can be released or solubilized from this fraction due to the effects of low molecular weight citric and oxalic acids excreted by plant roots and/or changes in soil pH and redox conditions.^{2,31,42,43}

Our results show that combination of SEP and XANES techniques provided vital information on the speciation of As in soils, which could be useful to better understand the lability, toxicity and phytoavailability of As in soils. XANES spectroscopy identified oxidation state ($\text{As}^{\text{V}}/\text{As}^{\text{III}}$) and mineral phases (e.g., scorodite, orpiment and coprecipitated or adsorbed forms) of As in soils, whereas the SEP was better in quantifying the highly mobile (Step-I and Step-II) As pools in soils.

Arsenic existed in soluble and mobilizable forms, and both As^{V} and As^{III} forms in historically contaminated soils; and the plant As uptake data reflect that As in these forms is phytoavailable. Soil properties especially Fe oxides and organic carbon contents are important in governing the speciation and phytoavailability of As in contaminated soils. Such detailed knowledge is vital for the reclamation and management of these As-contaminated soils.

■ ASSOCIATED CONTENT

Supporting Information. Three tables and three figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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