SILMARA COSTA SILVA

EVALUATION OF *Crambe abyssinica* Hochst. ex R.E.Fr. (Brassicaceae) FOR ARSENIC REMOVAL AND SPECIATION CHANGES IN AN OXISOL

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 09 de maio de 2017.

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ABSTRACT

SILVA, Silmara Costa, D.Sc., Universidade Federal de Viçosa, May, 2017. **Evaluation of *Crambe abyssinica* Hochst. ex R.E.Fr. (Brassicaceae) for arsenic removal and speciation changes in an Oxisol**. Adviser: Igor Rodrigues de Assis. Co-adviser: Luiz Eduardo Dias.

Arsenic (As) is widely distributed around the world. Despite its natural occurrence in some sites, As contamination is a concern for human health and the environment. Phytoremediation is an alternative to decontaminate As contaminated soils, by using hyperaccumulators or tolerant plants. This work aimed to evaluate the potential of *Crambe abyssinica* for As phytoremediation in response to different As and phosphorus (P) amendments in soil and to map the spatial distribution and speciation of As in As(III)- or As(V)-treated soil, the rhizosphere and plant parts of Crambe. Two different experiments were carried out. For the first experiment, a sandy Oxisol from the Brazilian Cerrado was incubated with P ($NH_4H_2PO_4$) and As (As_2O_3) dissolved in 1 mol KOH L^{-1} and oxidized to As(V), both at doses of 0, 50, 100, 150 or 200 mg dm^{-3} in a factorial experiment design. Three Crambe seedlings, previously germinated for 7 days, were transplanted to each greenhouse pot and allowed to grow for 105 days to the seed-production stage. Arsenic uptake by Crambe was evaluated, along with biomass production, As concentrations in roots, stems, leaves, and seeds; translocation and bioaccumulation factors; and seed germination. For the second experiment, the same type of soil from the first experiment was used for Crambe growth. To guarantee visualization of roots during the experiment, plants were cultivated in chambers in which the roots grew between two acrylic plates separated by 5 mm. The soil received 10.7 mmol L^{-1} solution of As(III) and As(V) separately, composing two different treatments. Plants were cultivated in a greenhouse for 20 days. For arsenic spatial distribution and speciation, samples were analyzed at the Synchrotron X-Ray Fluorescence beamline (SXRF) at the Brazilian Synchrotron Laboratory (LNLS), Campinas, SP. Samples were separated into leaves and the soil-rhizosphere system. The μ-SXRF analyses were performed using a white beam. After mapping an area of each sample, locations with higher As fluorescence intensity (hotspots) were identified, and μ-XANES spectra were collected using a monochromatic beam to identify arsenic species in the root-rhizosphere-soil continuum. After data collection, plants were harvested, separated into leaves and roots for trifluoracetic acid (TFA) extraction and speciation by HPLC-AFS. In general, we observed that biomass production decreased with increasing As for all P doses greater than the control treatments (without P and As addition). Arsenic concentration in plants increased with increasing P addition, reaching 759 mg kg^{-1} of As in the As150/P200 treatment (150 mg As dm^{-3} + 200 mg P dm^{-3}). Crambe grown in As-treated soils produced viable seeds with germination rates between 60 and 95 %, and containing less than 2 % of the total As in the plant, even for the highest As dose (As200). Our results also suggest that As is located mainly in veins of *Crambe abyssinica* leaves and most of the As in leaves is arsenite [As(III)]. In the root-rhizosphere system, differences between arsenic species in soil and rhizosphere were found for both samples, suggesting that Crambe roots change the rhizosphere in ways that induces changes in As speciation by arsenate reduction or promoting methylation, possibly to detoxify arsenic. The best As/P treatment for As content in Crambe shoot was As100/P200, which resulted in As removal of up to 9 kg ha^{-1} from soil. Besides accumulating high As amounts into its tissues, Crambe abyssinica plants showed TFs (Translocation Factors) and BFs (Bioconcentration Factors) of 5.09 and 61.11, respectively, suggesting that this species is potentially useful for phytoextraction of As from contaminated soils.

RESUMO

SILVA, Silmara Costa, D.Sc., Universidade Federal de Viçosa, maio de 2017. **Avaliação da espécie *Crambe abyssinica* Hochst. ex R.E.Fr. (Brassicaceae) para remoção e alteração de espécies de arsênio em Latossolo.** Orientador: Igor Rodrigues de Assis. Coorientador: Luiz Eduardo Dias.

O arsênio (As) ocorre naturalmente em diversos ambientes, porém as atividades humanas também podem mobilizar grandes quantidades deste elemento. A contaminação, seja por condições naturais ou antrópicas, é uma preocupação para a saúde humana e o meio ambiente. A fitorremediação é uma das alternativas mais viáveis para a descontaminação de solos contaminados por As, utilizando plantas hiperacumuladoras ou tolerantes. Este trabalho teve o objetivo de avaliar o potencial de *Crambe abyssinica* para fitorremediação de solo contaminado com As, em resposta a diferentes doses de As e fósforo (P) no solo, mapear a distribuição espacial e avaliar a especiação de As na rizosfera, na folha do Crambe e em solo tratado com As (III ou V). Dois experimentos foram realizados, sendo que no primeiro, um Latossolo arenoso do Cerrado Brasileiro foi incubado com P ($NH_4H_2PO_4$) e As (As_2O_3) solubilizado em KOH 1 mol L^{-1} e oxidado a As(V), ambos nas doses de 0, 50, 100, 150 ou 200 mg dm^{-3}, em esquema fatorial. Três plantas de Crambe, previamente germinadas, foram plantadas por vaso e o experimento foi conduzido por 105 dias em casa de vegetação. As avaliações realizadas foram: absorção de As pelo Crambe; produção de biomassa; concentração de As em raízes, caules, folhas e sementes; fatores de translocação e de bioacumulação; e taxa de germinação de sementes. No segundo experimento, o mesmo tipo de solo foi utilizado para o plantio de Crambe. Para garantir a visualização das raízes durante o experimento, as plantas foram cultivadas em placas acrílicas, de modo que as raízes cresceram entre duas placas separadas por 5 mm. O solo recebeu solução de 10,7 mmol L^{-1} de As(III) e As(V) separadamente, compondo dois tratamentos diferentes. As plantas foram cultivadas em estufa por 20 dias. Para avaliar a distribuição espacial de arsênio e especiação no solo e na rizosfera, as amostras foram analisadas na linha de luz de Fluorescência de

Raios – X (XRF) do Laboratório Nacional de Luz Sincrotron (LNLS), em Campinas, SP. As amostras foram separadas em folhas e sistema solo-rizosfera. As análises de µ-SXRF foram realizadas utilizando o feixe branco. Após o mapeamento, foram definidos os pontos com maior intensidade fluorescência de As (hotspots) e as análises de µ-XANES foram realizadas utilizando o feixe monocromático para identificar espécies de arsênio no contínuo raiz-rizosfera-solo. Após a coleta de dados, as plantas foram colhidas, separadas em folhas e raízes para extração em ácido trifluoroacético (TFA) e especiação por HPLC-AFS. A produção de biomassa diminuiu com o aumento de As para todas as doses de P maiores do que o controle (sem P). A concentração de arsênio nas plantas aumentou com a adição de P no solo, chegando a 759 mg kg^{-1} de As no tratamento As150/P200. O Crambe produziu sementes viáveis, com taxas de germinação entre 60 e 95%, e teores de As de no máximo 2% do total de As na planta, mesmo para tratamentos com maior dose (As200). Os resultados também sugerem que o As está localizado principalmente nas nervuras das folhas de *Crambe abyssinica* e a espécie de As mais comuns nas folhas é o arsenito [As(III)]. No sistema raiz-rizosfera, diferenças entre as espécies de arsênio no solo e rizosfera foram encontradas, sugerindo que a raiz de Crambe altera a rizosfera, induzindo mudanças nas espécies de As por redução do arsenato ou indução da metilação como mecanismo de tolerância ao As. O tratamento que apresentou melhor resultado com relação ao conteúdo de arsênio na parte aérea foi As100/P200, o que significa remoção de aproximadamente 9 kg ha^{-1} de As do solo. Além acumular grande quantidade de As nas folhas e caules, as plantas de *Crambe abyssinica* apresentaram TF (Fator de Translocação) e Fator de Bioacumulação (BF) de 5,09 e 61,11, respectivamente, sugerindo que esta espécie tem potencial para a fitoextração de As de solos contaminados.

GENERAL INTRODUCTION

Arsenic (As) is a toxic element that is naturally distributed throughout the Earth's crust as arsenic sulfides, metal arsenate [As(V)] or arsenite [As(III)]. However, human activities can also release As into water and soils by mining, fertilization, irrigation with contaminated groundwater, industrial wastes disposal, sewage-sludge treatment or pesticide use (Kabata-Pendias, 2011). Intake of inorganic arsenic for a long time can cause chronic arsenicosis (WHO, 2010) and other diseases. For this reason, As is one of the most hazardous elements to human health.

Soils in the world have around 7.2 mg kg^{-1} of As, ranging from 0.1 mg kg^{-1} until 97 mg kg^{-1} (Sparks, 2003). This content is widely distributed in different chemical species, depending on geological and environmental conditions. Some factors, such as pH and oxidation-reduction potential, along with clay content and phosphate presence, influence As mobility and bioavailability in soils.

In tropical soils, such as Brazilian Oxisols, arsenic mobility is limited as a consequence of Al and Fe oxyhydroxides predominance. However, soil water content, pH correction, or fertilization may enhance As mobility, and consequently, increase As availability to plants. Arsenic speciation also has an important role in As mobility. Anionic species such as AsO_2^-, AsO_4^{3-}, $HAsO_4^{2-}$ and $H_2AsO_4^-$, have greater mobility in soil, and As(III) species are more mobile than As(V) (Kabata-Pendias, 2011).

High arsenic concentrations in soil can be toxic to plants, causing several symptoms such as leaf wilting, cell plasmolysis, phosphorous deficiency, and inhibition of roots and plant growth. On the other hand, there are some terrestrial plants that are able to grow and take up high concentrations of arsenic in tissues. These plants have developed tolerance mechanisms, such as avoiding As uptake by roots or detoxifying As(V) to As(III) in order to complex it with phytochelatins and translocate it to aboveground parts.

In some plants, As is translocated to seeds, which represents a problem in terms of As contamination and human health. Some of these plants, such as rice, are important as human food around the world. In this case, As speciation and contents in rice grains (Abedin et al., 2002; Syu et al., 2015) are big concerns, due to the different toxicity as a function of As species. It is known that As(III) is more toxic than As(V), which are more toxic to As-methylated species, for plants and humans.

Given the risks associated with As contamination, different technologies for in-situ cleanup of As in soils and groundwater have been developed. Some of them are expensive, produce other secondary toxic waste, and are not sustainable (Huang et al., 2013). For these reasons, phytoremediation has been used as a cheaper remediation alternative, using plants to stabilize or remove contaminants from soils and water.

The success of phytoremediation depends on a plant's capacity to grow in a contaminated environment. Moreover, understanding how plants take up and store As is relevant to improving the technique and to achieve the best results. The most famous arsenic hyperaccumulator plant is a fern, *Pteris vittata*; however, this plant produces small amounts of biomass, which is a limitation for phytoremediation.

On the other hand, plants from Brassicaceae family, such as *Brassica juncea* and *Crambe abyssinica,* are reported to be As tolerant species and high biomass producers. After hydroponic experimental results, *Crambe abyssinica*, has lately been considered as a suitable plant for As phytoremediation (Artus, 2006). This finding suggests that Crambe also has tolerance to high As concentrations in soil, and that this plant makes changes in the rhizosphere in order to modify As species before uptake. The general objective of this work was to evaluate the potential of *Crambe abyssinica* for phytoremediation of As.

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LITERATURE REVIEW

Arsenic in the Environment

Arsenic is a toxic metalloid and remains for a long time in the environment, affecting soil quality and properties, especially its fertility (Garg & Singla, 2011). This element belongs to family 15 of the periodic table and occurs in different forms in the environment. Anionic forms, such as AsO_2^-, $HAsO_4^{2-}$, $H_2AsO_3^{2-}$ predominate in soil solution (Kabata-Pendias & Sadurski, 2004). The most common As(V) species are $H_2AsO_4^-$ (at common soil pH between 4 and 6.94) and $HAsO_4^{2-}$ (at common soil pH between 6.94 and 8.5) whereas As(III) occurs mostly as H_3AsO_3, under low pH and Eh conditions (Kabata-Pendias, 2011; Lu and Zhu, 2011).

Arsenic mobility is related to soil chemical, physical and mineralogical properties. Soils with abundant Fe and Al oxides and hydroxides usually retain more As anions, particularly as As(V) (Alloway, 1995). In soils with high pH, the predominance of negative pH dependent charges favors arsenic mobility.

Due to the similar atomic characteristics, phosphate and arsenate have analogous behavior in soil. Both occur mainly as oxyanions and can be adsorbed to soil particles, especially in positively charged soils (Bolan et al., 2013; Garg & Singla, 2011). Depending on soil characteristics, such as mineralogy and clay content, arsenate and phosphate compete for soil adsorption sites, changing As and P concentration in soil solution and, consequently, their availability for plants.

Arsenic and Phosphorous in plant

The main phosphate source for plants in agricultural soils is fertilization. In addition to promoting plant growth, phosphate may also change As reactions in soils and plants. The extraction of As by plants depends mainly on the P/As molar ratio in soil (Santos et al., 2010), the time of phosphate application (Santos et al., 2008) and plant genotype (Zhu et al., 2006).

Phosphate can decrease As absorption by plants due to the competition in phosphate transporter systems and, consequently, relieve As toxicity effects (Palma et al., 2013). This mechanism results in continuous production of biomass, without increasing the arsenic concentration in shoots, and limits the transfer of As to the seeds (Pigna et al., 2010). On the other hand, higher arsenic solubility and bioavailability may occur due to the ability of phosphate to displace the adsorbed arsenic in the soil sorption complex (Kabata-Pendias, 2011; Palma et al., 2013).

Arsenic uptake occurs by different routes, depending on As species availability in soil solution. Generally, arsenite is taken up by glycerol transport channels, whereas As(V), which is predominant in tropical soils, is transported to shoots by phosphate transport routes (Bolan et al., 2013).

Due to this similarity, studies were carried out with different plant species to evaluate the influence of phosphate on arsenic uptake (Cipriani, 2011; Grifoni et al., 2014; Jiang et al., 2014; Lu et al., 2010; Pigna et al., 2010; Vázquez et al., 2008). In these works, the results suggested that phosphorous presence in the soils or the greater capacity of P absorption by the plants, decreases arsenic levels in plant tissues. The same effect was observed in grains of wheat (Pigna et al., 2010) and rice (Lu et al., 2010). On the other hand, in an experiment performed with *Brassica juncea*, Grifoni et al. (2014) observed that both control plants (without P added), as well as, those receiving P, did not exhibit As-toxicity symptoms and there was no change in biomass production in response to the phosphate treatments.

The predominant forms of As in plant tissues are inorganic species of As(V) and As(III), but there is also a small amount of organic species (Palma et al., 2013). Among the organic species, the most common are methylated forms: dimethylarsinic (DMA) and monomethylarsonic (MMA), which are less toxic than inorganic species. The absorption factor of roots of different plants can be five times higher for As(V) than for DMA and 2.5 times than for MMA (Raab et al., 2007). Thus, a mechanism of detoxification by some living organisms is the methylation of inorganic As species within the body (Barra et al., 2000).

Detoxification mechanisms can occur both within the plant or in the rhizosphere, the interface between roots and soil where chemical and biological processes make changes on arsenic species before plant uptake (Kidd et al., 2009; Zhao, et al., 2009). Once present in plant tissue, arsenic may inhibit the activity of some proteins and uptake of essential elements, causing oxidative stress due to the formation of free radicals. It also competes with and replaces phosphate in ATP. From this substitution, ADP-As(V) is formed, which is unstable and compromises the energy flow of cells (Bolan et al., 2013).

Arsenic toxicity is usually noticed in plants that grow in mining environments and in soils that receive pesticides or contaminated sewage sludge. Toxicity symptoms may range from withered leaves, violet coloration, and root discoloration, to cell plasmolysis. However, the most common effect is decreased biomass production.

Plants that accumulate arsenic in their tissues are called either hyperaccumulators or tolerant plants. Those that take up As (or other trace elements) above 1000 mg kg^{-1} and translocate it to shoots are called hyperaccumulators (Kabata-Pendias, 2011). Arsenic tolerant plants, on the other hand, are those that accumulate more As than most plants, but <1000 mg As kg^{-1}, like hyperaccumulator, and also tend to restrict the transfer of arsenic from soil to plant and from root to shoot (Fitz & Wenzel, 2002). Due to these characteristics, both tolerant and hyperaccumulator plants have great importance for phytoremediation of contaminated areas.

Phytoremediation of arsenic contaminated areas

Soil degradation processes can be aggravated by the destruction of vegetation cover in contaminated areas and can promote erosion and leaching of contaminants to the groundwater (Melo et al., 2009). For these reasons, it is necessary to promote the remediation of these soils.

One of the major problems related to remediation of contaminated soils is the high cost involved. The lowest cost technique, compared to conventional ones, is phytoremediation. This technique uses plants to remove, transform or immobilize toxic elements present in soil, sediments, ground and surface water, and eventually into the atmosphere. It is a tool currently used for treatment of several classes of contaminants, including heavy metals (Susarla et al., 2002) and metalloids such as arsenic.

In phytoremediation, plants act as bio-pumps to remove contaminants and water from the subsoil. This process can be divided into phytostabilization, phytoextraction, and phytofiltration or rizofiltration (Kabata-Pendias, 2011):

- Phytostabilization: plants decrease the mobility and bioavailability of contaminants in the environment by immobilization preventing their migration.

- Phytoextraction: plants remove metals from soil and accumulate them in aboveground parts that will later be removed.

- Phytofiltration or rizofiltration: roots are responsible for absorbing effluent metals.

Currently, an urgent demand for phytoextraction is to increase the variety of plants that accumulate soil metals and to develop technologies suitable for the use of the contaminant-laden plant materials (Kabata-Pendias, 2011). Many hyperaccumulator plants have low biomass production or low growth rate, due to the allocation of most of the energy to the necessary mechanisms of adaptation to the high concentration of metals in the tissues. With some exceptions, plants with greater biomass accumulation rates are generally sensitive to metals and accumulate only small amounts in the aerial part (Artus, 2006). Therefore, these characteristics may limit their use in phytoextraction processes.

Since phytoremediation requires the use of plant species, acidity correction and fertility improvement of these soils are necessary. However, the addition of fertilizers to contaminated areas can influence soil dynamics (Klaber & Barker, 2014; Pigna et al., 2010) and, these contaminants may become more mobile due to the type of added ion and, also, of the predominant charges in these soils (Bolan et al., 2013).

Crambe abyssinica

There are several studies aimed at the identification of arsenic-tolerant species. However, few species are known to tolerate or hyperaccumulate this element. Among the exceptions, we can highlight *Pteris vittata* (Ma et al., 2001), *Pityrogramma calomelanos* (Francesconi et al., 2002), *Brassica juncea* (Pickering,

2000) and some herbaceous leguminous plants such as *Crotalaria spectabilis* (Dias et al., 2009). These species, in general, have specific mechanisms for As accumulation in tissues, related in general, to the performance of antioxidative enzymatic mechanisms (Felipe et al., 2009). According to Prasad & Hagemeyer (1999), the most significant toxic effects of arsenic in plants are related both to the competition of exchange sites with essential metabolites and to the occupation of sites of essential groups such as phosphate and nitrate.

The selection of species that is not only tolerant to As, but also to other unfavorable environmental conditions (e.g., salinity, high electrical conductivity, low organic matter, heavy-metal contamination) is fundamental for the success of soil phytoremediation. In this context, one of the species that has potential for As phytoremediation is *Crambe abyssinica* Hochst, an oleaginous plant belonging to the Brassicaceae family (Lalas et al., 2012).

Crambe is an exotic species, originally from the Mediterranean region. It has been studied as an alternative crop in the Brazilian bioenergy program, since it seed produces oil with potential for biofuels production. Its oil is distinguished from the others basically by the high content of erucic acid, which has many uses in the industry. The oil extracted from crambe's seeds is used for the production of rubber, plastics, adhesives, electrical insulators, lubricants and cosmetics, among other uses (Colodetti et al., 2012). Moreover, crambe seeds are not used for human or animal consumption, due to its non-palatability.

Crambe does not grow very well in shallow soils, but has good resistance to salinity (Falasca et al., 2010). This culture has a fast reproductive cycle (ninety days), with high biomass production, which is a good characteristic for the phytoremediation of contaminated areas (Zulfiqar et al., 2011). Preliminary hydroponic studies have shown that this species is tolerant to As (Paulose et al., 2010) and other heavy metals, being able to accumulate higher levels of As and Cd than other species of the same family (Artus, 2006).

The hydroponic system is used in arsenic tests to suppress the effects of chemical complexation and the low bioavailability of As that can occur in soils

(Artus, 2006). However, this type of experiment overestimates the importance of the absorption kinetics of the plant in question and the processes involving the soil-plant relationship (Fitz & Wenzel, 2002).

Due to the lack of knowledge about crambe cultivation in As-contaminated soil, this work aimed to elucidate crambe's behavior in an Oxisol amended with As in different amounts and to evaluate rhizosphere changes as a function of As presence in this soil.

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CHAPTER 1: Arsenic phytoremediation by *Crambe abyssinica* as affected by soil As and P

Abstract

Arsenic (As) contamination of soils, water, and food crops is a concern for human health and the environment. Phytoremediation by As hyperaccumulators or tolerant plants is an important technique to decontaminate soils. Our objective was to evaluate the potential of *Crambe abyssinica* to remove (phytoextraction) or stabilize (phytostabilization) As from soil in response to different As and phosphorus (P) amendments. A sandy Oxisol from the Brazilian Cerrado was incubated with As and P, both at doses of 0, 50, 100, 150 or 200 mg dm^{-3} (As0 – As200 and P0 – P200) in a factorial design. Arsenic uptake by Crambe was evaluated, along with biomass production, As concentrations in roots, stems, leaves, and seeds; translocation and bioaccumulation factors; and seed germination. Plant biomass production decreased with increasing As for all P doses greater than the control (without P). Arsenic concentration in plants increased with increasing P addition, reaching maxima 729 mg As kg^{-1} of shoots and 982 mg kg^{-1} whole plant for the As150/P150. This result suggests that phosphate improves As removal from soil by Crambe. Crambe grown in As-treated soils produced viable seeds with high germination rates. For the As200/P150 treatment, As in the seeds was greatest at 1.6 % of the total plant As for this treatment. Given that Crambe accumulates nearly as much As as hyperaccumulator plants and also has high biomass production, Crambe should be a viable plant for phytoextraction of As from contaminated soils in different climates.

Introduction

Worldwide, soil arsenic (As) concentrations range from 0.1 to 1000 mg kg^{-1} (USEPA, 2000). This element is a food and groundwater contaminant (Escobar et al., 2006), and is the highest priority toxic contaminant of concern to human populations (ATSDR, 2015).

To mitigate human exposure to toxic substances, contaminated soils are remediated by techniques such as extraction or *in-situ* stabilization as alternatives to excavation and landfilling. Specific approaches include soil vapor extraction, soil leaching or ex-situ washing, and solidification. Most of these approaches are expensive and require high technology systems (Cunningham et al., 1995; Tauqeer et al., 2016; Wuana & Okieimen, 2011). On the other hand, phytoremediation, which uses plants for soil remediation, is an inexpensive technique and depends mostly on plant responses at the site rather than on high technology.

The success of phytoremediation depends on plant tolerance to high concentrations of toxic elements in soils. To improve plant tolerance, synergistic chemical treatments such as P amendments of As contaminated soils can be used in phytoremediation (Tu & Ma, 2003). Arsenic and P have similar properties because of their atomic structures, and are commonly found as oxyanions (arsenate and phosphate) in Oxisols (Manning & Goldberg, 1996; Arai & Sparks, 2007; Violante & Pigna, 2002). Both arsenate and phosphate can be adsorbed by soil colloids and are less dissolved in soil solution. However, due to their competition for the same adsorption sites in soil, one element can increase the other's availability when they are present in the same soil (Smith et al., 2002). In plants, arsenate can be taken up by phosphate uptake systems and also compete with phosphate in cytoplasm (Quaghebeur & Rengel, 2003). In cells, arsenic can replace phosphate during ATP formation, producing ADP-As, which is an unstable complex in the electron transport chain (Ullrich-Eberius et al., 1989).

Hyperaccumulator plants are able to remove As from soil and store it in aboveground biomass at concentrations above 1000 mg kg^{-1}. This characteristic is important for soil remediation, enhancing As extraction and soil decontamination capacities. One of the most famous hyperaccumulator plants is *Pteris vittata* (Ma et al., 2001), which accumulates high amount of As in fronds (up to 23000 mg kg^{-1}). Other As hyperaccumulators species are *Pityrogramma calomelanos* (Francesconi et al., 2002) and *Brassica juncea* (Pickering et al., 2000).

Arsenic-tolerant plants are able to grow in As-contaminated soils. Some examples of tolerant plants are *Andropogon scoparius*, *Agrostis castellana*, *Agrostis capillaris* and *Holcus lanatus* L. (De Koe & Jaques, 1993; Meharg & Macnair, 1991; Rocovich & West, 1975). Even though these plants typically accumulate less than 1000 mg kg^{-1} of arsenic in their aboveground tissues, they can immobilize As in roots, avoiding its translocation to shoots.

Recently, *Crambe abyssinica* was reported as a potential plant for As remediation (Artus, 2006; Paulose et al., 2010). This species is native of Mediterranean regions (Colodetti et al., 2012) and belongs to the Brassicaceae family (Lalas et al., 2012). Crambe plants produce oilseeds that are used in most of the lubricant and cosmetic industries. Also, Crambe has been studied as an alternative to biofuel production, due to the high content of oil in its seeds (approximately 35 %) (Lazzeri et al., 1994).

Crambe abyssinica can be cultivated as a spring-summer or as a winter crop, and under high salinity soil conditions (Lazzeri et al., 1994; Ionov et al., 2013; Falasca et al., 2015). Thus, Crambe spends 90 days to achieve seed maturity, and produces high biomass, approximately 3.0 t ha^{-1} (Heinz et al., 2011), which are important characteristics for soil phytoremediation (Mcgrath & Zhao, 2003; Zulfiqar et al., 2011).

Although researchers have found that Crambe is tolerant to high concentrations of cadmium and arsenic (Artus, 2006; Paulose et al., 2010), these studies were carried out under semi-controlled conditions in hydroponic solution. There are some limitations to up-scaling their findings to realistic soil conditions, due to the overestimation of the effects of uptake kinetics in plant and the importance of soil-plant interactions in As mobility (Fitz & Wenzel, 2002). The objective of this study was to evaluate the potential of *Crambe abyssinica* to remove or stabilize As from soil in response to different As and P levels.

Material and Methods

Experimental design

In order to evaluate As and P interactions in *Crambe abyssinica*, five concentrations of each element (0, 50, 100, 150 and 200 mg dm^{-3}) were distributed in randomized block design, factorial scheme, with four replicates, totalizing 100 experimental units.

Greenhouse experiment

A sandy Oxisoil from the Brazilian Cerrado with low CEC, low plant-available P, and no available As was used for Crambe growth (Table 1). Soil was sieved through a 4-mm screen and distributed in pots of 5 dm^3 without holes to avoid As and P loss via water drainage.

Table 1. Chemical and physical characteristics of the soil used in this study.

Property	Soil
/1pH (1:2.5)	4.36
Organic matter content (dag kg^{-1})	1.01
/2Cation exchange capacity – CEC (cmol$_c$ dm^{-3})	0.99
Remaining phosphorous (mg L^{-1})	32.4
/3Available phosphorous (mg dm^{-3})	0.33
/4Available arsenic (mg dm^{-3})	0.00
Sand (%)	79.0
Silt (%)	2.00
Clay (%)	19.0
Density (g cm^{-3})	1.06

/1pH measured in 1:2.5 (soil:water) ratio. /2at soil pH. /3Mehlich I extraction. /4Mehlich III extraction.

In order to adjust the soil pH to 6.0, $CaCO_3$ and $MgCO_3$ (4:1 ratio) were mixed with the bulk soil before splitting into pots and then incubated for 15 days. An As(V) treatment solution was prepared by dissolving As_2O_3 in KOH (1 mol L^{-1}) solution, which was oxidized by adjusting the pH and oxidation-reduction (redox) potential with HCl and H_2O_2 to pH > 5.5 and Eh > 650 mV, respectively, to obtain only As(V) in solution. After incubation with arsenic solutions for 45 days, phosphate was provided as $NH_4H_2PO_4$. Nitrogen and potassium were added using NH_4NO_3 and KCl, respectively. Other macro and micronutrients were provided as recommended by Alvarez V. (1974).

Three crambe seedlings, previously germinated for 7 days, were transplanted to each pot. Soil samples were collected from each pot after transplanting to measure Mehlich III extractable As and P in soil (Mehlich, 1984). Plants were grown to seeding in the greenhouse for 105 days, from February to May of 2015, then harvested. The plants were watered twice a day, or as needed.

All leaves and stems were collected, rinsed with deionized water, dried at 60 ºC for 72 h, weighed for biomass production, and ground for As and P analysis. Roots were previously separated from soil, washed thoroughly with tap water and then rinsed with 0.01 mol L^{-1} of HNO_3 solution followed by three rinses with deionized water. Subsequently, roots were dried at 60 ºC for 72 h, ground and weighed for As and P determination. Seeds were collected, refrigerated between 6 and 10 ºC, and ground before chemical analysis.

Chemical analysis

For As and P determination, approximately 0.5 g of grounded plant samples (grains, leaves, stems and roots) were mixed with 9 mL of a nitric-perchloric acid and set aside for 16 h for pre-digestion. After this period, samples were digested on a hotplate at 160-180 ºC. The ratio of nitric to perchloric acids was 3:1 for leaves, stems and roots, and 5:1 for seeds digestion due to the high oil content in seeds. The extracts were analyzed by ICP-OES. Detection limits were calculated from multiple analyses of blank solutions based on equation 1,

$$DL = 3 \times \text{Stdev}([\text{Blank}])/S \qquad (1)$$

where DL (mg L^{-1}) is the detection limit, Stdev (Blank) is the standard deviation of element concentration of all blank samples, and S is the slope of a calibration curve calculated by emission intensity for each concentration.

Seed germination

Fifty seeds of each experimental unit (except treatments with 0 mg dm^{-3} of P) were placed on Germitest® paper previously moistened with distilled water at a volume corresponding to 2.5 times the dry weight of the paper. The paper and the seeds were placed inside an acrylic box (11 x 11 x 2.5 cm). In order to cover the seeds, another layer of Germitest® was place above them. The boxes were sealed and incubated in a germination chamber at 20 ºC, with alternating light-dark periods of 12 hours (Santos & Rossetto, 2013). Germinated seeds were counted after 4 and 7 days, based on established Brasil (2009) criteria.

Statistical analysis

All measured variables were submitted to analysis of variance (ANOVA). and regression analysis was performed to obtain models relating P to As addition in soil.

Results

Generally, plants did not show As toxicity symptoms, and biomass and seed production were exceptionally low only in the P0 treatments (Fig. 1).

Extractable arsenic and phosphorus concentrations

Table 2 shows regression functions adjusted for Mehlich III extractable As and P in soil after 45 days of As incubation. After planting crambe seedlings, available As and P in soil solution increased as more As and P were added to the soil samples. No significant interaction between the availability of these two elements in soil was observed. Both arsenic and phosphorous curves showed linear trends, resulting in more available As and P to plants with increasing dose.

Table 2. Mehlich-III extractable soil As and P after 45 of incubation as a function of As and P additions.

Variable	Regression function	R^2
Extractable As (mg dm^{-3})	-3.80 + 0.23**As	0.84
Extractable P (mg dm^{-3})	-3.85 + 0.31**P	0.81

**; * = significant at <0.01 and <0.05, respectively.

Shoot dry matter

Dry matter in shoots (seeds, leaves and stems) decreased with increasing As for all P treatments (Fig. 1). However, when more phosphate was applied to soil (P200), more dry matter was produced in shoots of plants growing in As150 and As200 concentrations. The decreasing of dry matter production for P200 treatments was also less intense than others (except for P0), which can be inferred by the less negative slopes of the P200 curves of -0.0319, -0.0091, and -0.0680, compared with slopes <-0.0410, <0.0222, and <-0.0840 for non-zero treatments of seeds, leaves and stems, respectively.

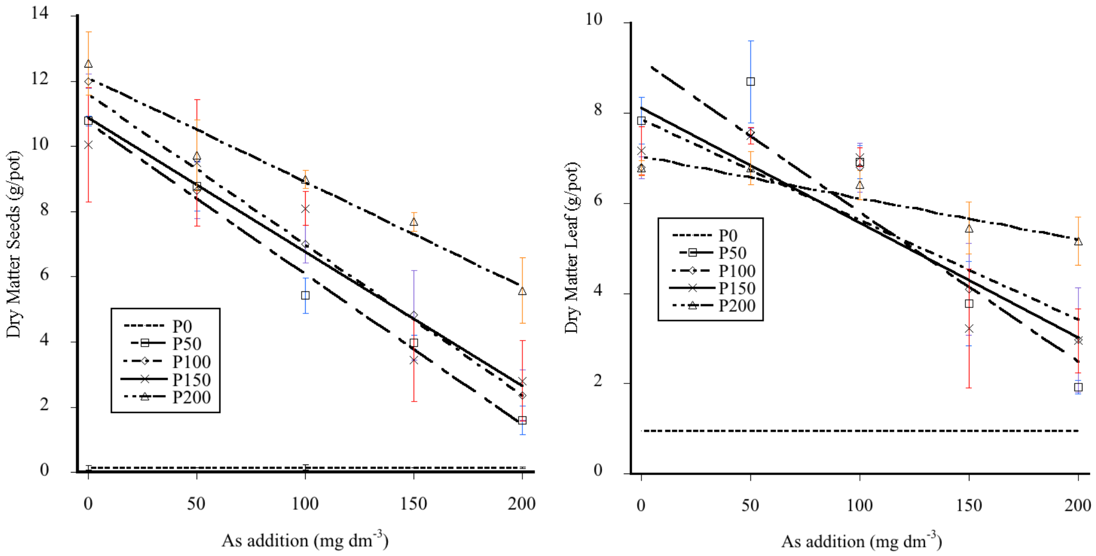
Arsenic and phosphorous uptake into *Crambe abyssinica*

Arsenic concentrations in seeds, stems and roots increased linearly with increasing As additions to soil. On the other hand, As concentrations in leaves increased to approximately 100 mg As dm^{-3} soil for P50, P100, P150, and P200 treatments (Fig. 2), then decreased at As concentrations of 150 and 200 mg dm^{-3}.

The As concentrations in leaves, stems and seeds was higher than in roots for all As treatments other than the control, and higher concentrations of As in these above-ground plant tissues were observed in treatments with more P (P150 and P200). The treatment that produced the highest plant As concentration was the As150-P150 treatment. The total plant As concentration in this treatment reached 729 mg kg^{-1} in shoots and 982 mg kg^{-1} in the whole plant. For seeds, the highest As concentration (mg kg-1) was observed for the As200-P150 treatment, which represented 1.6 % of the total As in the plant.

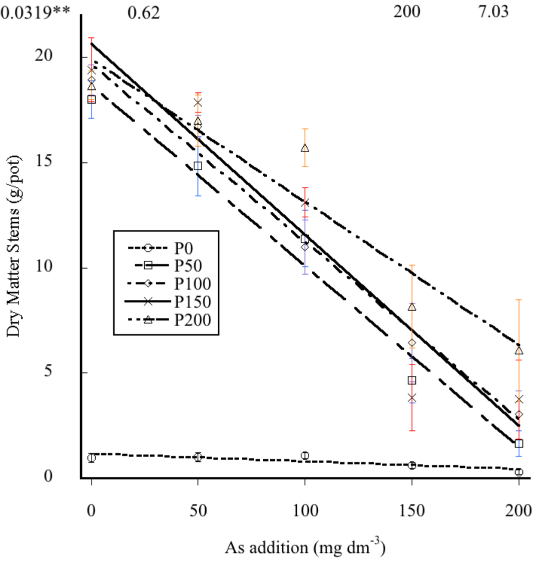
A comparison of P and As uptake into Crambe shoots only showed that As generally increased and P typically decreased with increasing As additions (Fig. 3). However, similar to for leaves (Fig. 2) As concentration decreased at additions >150 mg dm^{-3} for the P200 treatments, as a consequence of decrease in As leaves concentration for the same treatments. Also, P concentrations in shoots remained constant for the P0 and P50 treatments and decreased linearly with increasing As concentration for P treatments between P100 and P200. The maximum inhibition of uptake was observed in P200 treatments, resulting in less P uptake due to high As in soil (Fig. 3).

Phosphorus contents of shoots, i.e., amounts of P in plants per pot (mg/pot), accounting for overall biomass, decreased linearly with increasing soil As treatment (Fig. 3). However, shoot P contents increased overall with increasing P additions, regardless of the soil As addition, presumably because soil P availability increased. For P50, P100 and P150, As contents (mg/pot) in shoots were curvilinear with As additions, increasing up to the As100 treatment, then decreasing for greater As treatments. For the As 100 and P200 treatment, the As content in shoots reached 21.54 mg/pot. Plant shoots accumulated increasing amounts of As as applied soil P increased, and our data in Fig. 3 suggest that P additions greater than our highest P dose of 200 mg dm^{-3} soil could further increase As uptake into *Crambe abyssinica*. This hypothesis should be verified in future experiments.



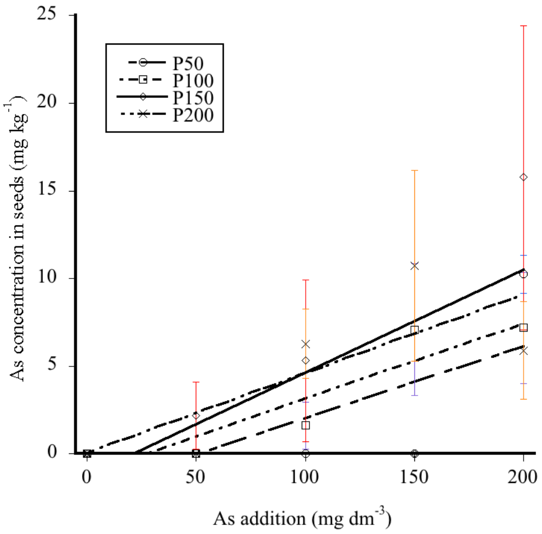
P content	Intercept	As	R^2 (adjusted)
0	-	-	Mean = 0.15
50	10.75	-0.0463**	0.90
100	11.58	-0.0461**	0.77
150	10.88	-0.0410**	0.45
200	12.01	-0.0319**	0.62

P content	Intercept	As	R^2 (adjusted)
0	-	-	Mean = 0.96
50	9.18	-0.0335**	0.66
100	7.86	-0.0222**	0.43
150	8.11	-0.0254**	0.49
200	7.03	-0.0091**	0.32

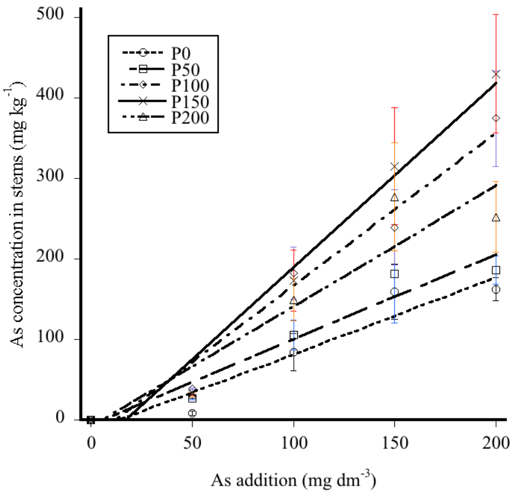


P content	Intercept	As	R^2 (adjusted)
0	1.15	-0.0035**	0.27
50	18.68	-0.0857**	0.86
100	19.69	-0.0840**	0.84
150	20.65	-0.0906**	0.78
200	19.92	-0.0680**	0.64

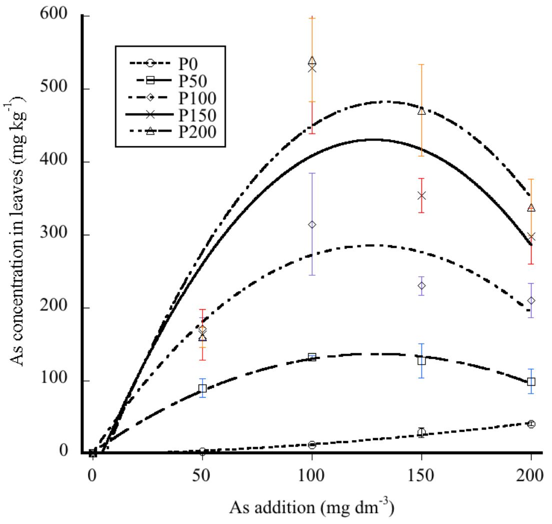
Figure 1. Mean (n= 4) dry weights of Crambe leaf, seed, and stem production as a function of As dose for different levels of applied P (P0, P50, P100, P150, and P200). For linear-regression models, **=significant at <0.01; *= significant at <0.05; °= significant at <0.10.



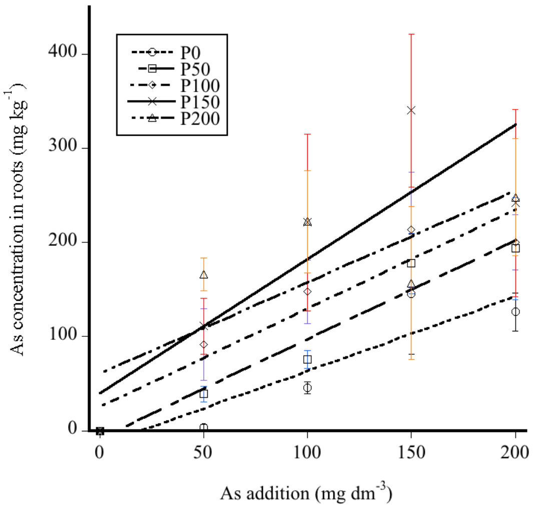
P content	Intercept	As	R^2 (adjusted)
0	-	-	-
50	-2.05	0.0410**	0.44
100	-1.11	0.0430*	0.25
150	-1.21	0.0587°	0.10
200	0.08	0.0451*	0.16



P content	Intercept	As	R^2 (adjusted)
0	-12.36	0.9508**	0.69
50	-5.26	1.0525**	0.57
100	-22.91	1.8979**	0.74
150	-38.39	2.2845**	0.70
200	-7.86	1.4985**	0.59

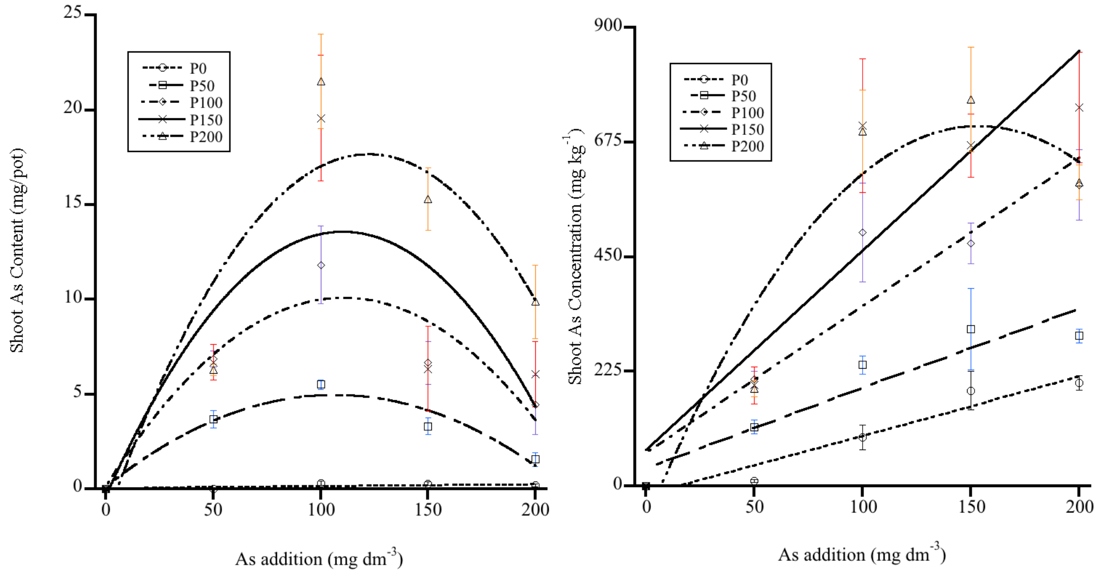


P content	Intercept	As	As^2	R^2 (adjusted)
0	-1.08	0.0585	0.0008°	0.81
50	2.10	2.0869**	-0.0081**	0.70
100	2.14	4.4474**	-0.0174**	0.60
150	-28.37	7.1672**	-0.0280**	0.59
200	-43.59	7.8926**	-0.0296**	0.71



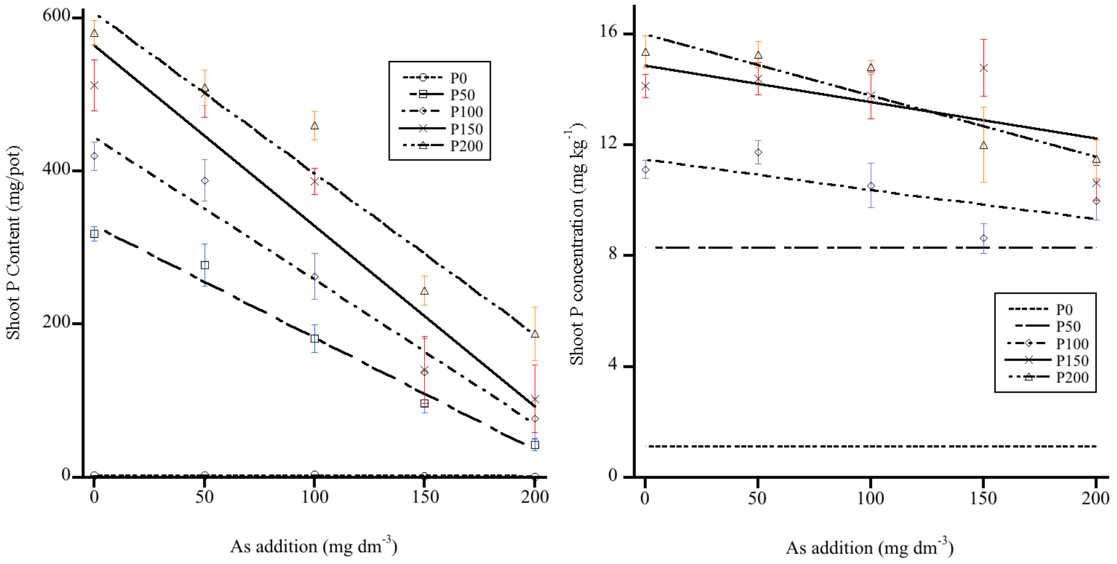
P content	Intercept	As	R^2 (adjusted)
0	-14.62	0.79**	0.39
50	-8.02	1.05**	0.58
100	26.07	1.04**	0.43
150	40.34	1.42*	0.25
200	61.30	0.97*	0.22

Figure 2. Mean (n= 4) As concentrations in Crambe leaf, seed, and stem as a function of As dose for different levels of applied P (P0, P50, P100, P150, and P200). For linear- and polynomial-regression models, **=significant at <0.01; *= significant at <0.05; °= significant at <0.10.



P content	Intercept	As	As^2	R^2 (adjusted)
0	0.03	0.0013*	-	0.16
50	0.14	0.0908**	-0.0004**	0.80
100	0.18	0.1792**	-0.0008**	0.57
150	-0.34	0.2524**	-0.0011**	0.39
200	-1.56	0.3143**	-0.0013**	0.66

P content	Intercept	As	As^2	R^2 (adjusted)
0	-17.38	1.1666**	-	0.74
50	35.20	1.5636**	-	0.61
100	65.36	2.8982**	-	0.68
150	71.86	3.9171**	-	0.62
200	-67.74	10.0910**	-0.0329**	0.75



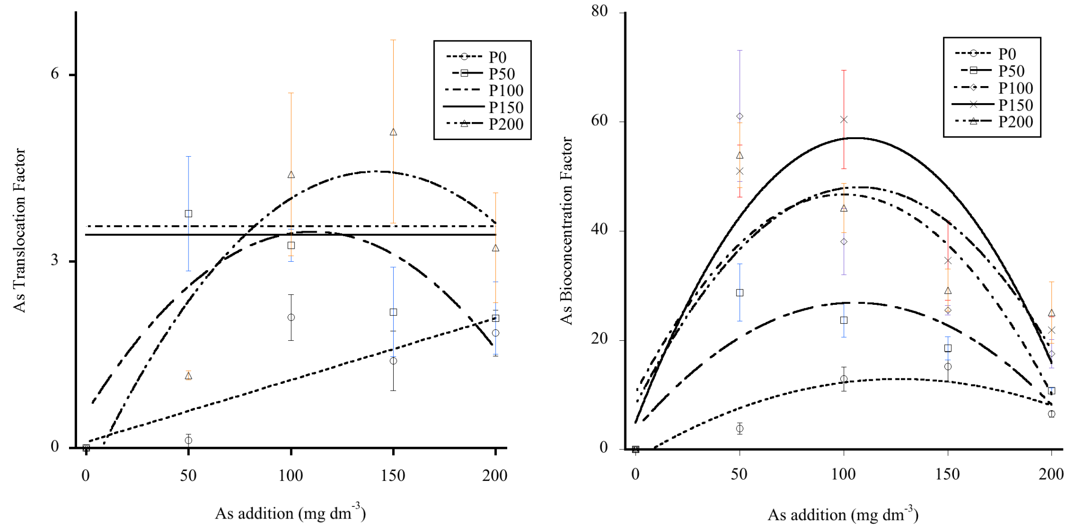
P content	Intercept	As	R^2 (adjusted)
0	2.89	-0.0082**	0.23
50	329.55	-1.46**	0.89
100	444.00	-1.87**	0.79
150	564.78	-2.36**	0.77
200	606.64	-2.10**	0.85

P content	Intercept	As	R^2 (adjusted)
0	-	-	Mean = 1.12
50	-	-	Mean = 8.29
100	11.48	-0.0108*	0.19
150	14.86	-0.0131*	0.15
200	15.98	-0.0220**	0.44

Figure 3. Mean (n= 4) As and P contents and concentrations in crambe shoots as a function of As dose for different levels of applied P (P0, P50, P100, P150, and P200). For linear- and polynomial-regression models, **=significant at <0.01; *= significant at <0.05; °= significant at <0.10.

Arsenic translocation and bioconcentration factors

Translocation factor (TF) is the As concentration ratio in shoots to roots (Luongo & Ma, 2005). As previously shown, As concentrations in shoots in our P200 treatment increased up to an input of 150 mg As dm^{-3}, and decreased at greater inputs (Fig. 3). The same tendency was observed in TF for the P200 treatment (Fig. 4). In P0 and P150 treatments, TF increased with As in soil. The maximum TF (5.09) was observed for the As150 and P200 treatments.



P content	Intercept	As	As^2	R^2 (adjusted)
0	0.10	0.0099**	-	0.39
50	0.56	0.0256**	-0.0002*	0.28
100	-	-	-	Mean = 3.56
150	-	-	-	Mean = 3.43
200	-0.53	0.0699*	-0.0002°	0.37

P content	Intercept	As	As^2	R^2 (adjusted)
0	-1.72	0.2305**	-0.0009**	0.57
50	3.64	0.4407**	-0.0021**	0.53
100	10.31	0.7289**	-0.0036**	0.34
150	4.87	0.9853**	-0.0046**	0.63
200	8.05	0.7447**	-0.0035**	0.47

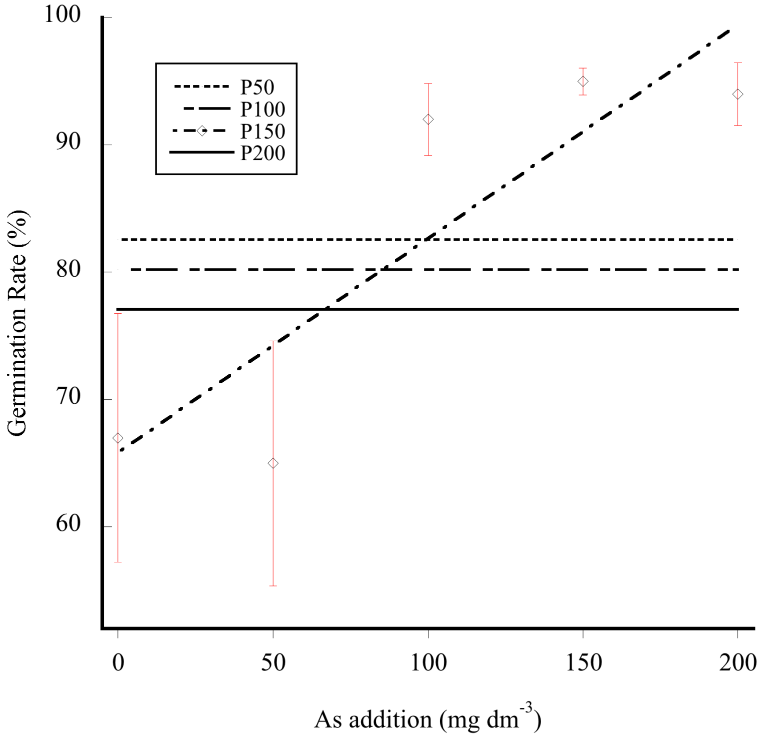
Figure 4. Mean (n= 4) As translocation factor from roots to shoots and As bioconcentration factor as a function of As dose for different levels of applied P (P0, P50, P100, P150, and P200). For linear- and polynomial-regression models, **=significant at <0.01; *= significant at <0.05; °= significant at <0.10.

The Bioconcentration Factor (BF) is the ratio of As concentration (mg kg^{-1}) in plant tissue to the As concentration (mg dm^{-3}) in soil (Lessl et al., 2015). To calculate BF, we used Mehlich-III extractable As as a measure of soil As that is more readily solubilized, and potentially more available to plant. This value was used in order to obtain consistent results to the As that plants can, in fact, uptake from soil and accumulate in tissues.

The highest values of BF were 61 and 60 observed in plants growing in soils treated with As50/P100 and As100/P150, respectively (Fig. 4). Regardless of P treatment, As accumulation in tissues decreased for soil additions greater than 100 mg dm^{-3} of As (As150 and As200).

Germination test

Average germination rates of seeds produced by *Crambe abyssinica* ranged from 61-95 % for all treatments. For P150 treatment, it was observed increasing germination rate with increasing As addition, while, for other P treatments, germinations rates remained constant (Fig. 5).



P content	Intercept	As	R^2 (adjusted)
0	-	-	-
50	-	-	Mean = 82.57
100	-	-	Mean = 80.20
150	65.80	0.168**	0.37
200	-	-	Mean = 77.09

Figure 5. Mean (n= 4) germination rates of crambe seeds as a function of As dose for different levels of applied P (P0, P50, P100, P150, and P200). For linear-regression model, **=significant at <0.01; *= significant at <0.05; °= significant at <0.10.

Discussion

Arsenic availability in soil

Soil extractable As concentrations were 0, 5, 15, 27, and 43 mg kg^{-1} for As0, As50, As100, As150, and As200 addition, respectively. According to Sheppard (1992), the mean threshold of arsenic toxicity in plants growing in a sandy soil is 40 mg kg^{-1}. In my study, no toxicity effects, such as severe cellular damage or injury in leaves, were observed in plants grown on soils receiving treatments of 200 mg As dm^{-3} and >50 mg P dm^{-3}. These results suggest that Crambe tolerates more As more than typical plants, depending on soil conditions and P supply.

Despite the known interactions between As and P in soil, our ANOVA and regression results showed no interaction between Mehlich-III extractable P and As in soil (Table 2), suggesting that As availability to crambe was not limited by P, but that plant arsenic tolerance mechanisms were activated in order to mitigate As effects in plant tissues.

As and P concentration in plants had different accumulation pattern

Arsenic has no known biological function in plants, however, some species such as *Pteris vittata* show increasing growth with As uptake (Lessl et al., 2015). *Crambe abyssinica* had decreased growth with increasing soil As addition, which indicates that As amendments changed plant metabolism due to As phytotoxicity.

Arsenic uptake by Crambe into different plant structures was directly proportional to As concentrations in soil, except for leaves. Arsenic concentration in leaves decreased above the As100 treatment, which can be explained by important plant functions performed by leaves such as photosynthesis (their primary function) (Gal et al., 2012), respiration, and transpiration. As a tolerance mechanism, after this point (As100), plants accumulated more As in stems than in leaves. These results suggest that the plant delocalized As from leaves to other plant parts when the leaf concentration reached some threshold.

Arsenic and P uptake varies depending on the plant species, tissue and growing conditions (Tu & Ma, 2003). For *Crambe abyssinica*, greater soil P concentrations resulted in more As accumulation in plant tissues. The results showed more As uptake and also dry matter production due to phosphate in soil (e.g., for the P200 treatment). Similar behavior was observed in *Brassica juncea*, which belongs to the same family of *Crambe abyssinica*. In As contaminated soil, increasing soil P stimulated As uptake by *Brassica juncea* (Bolan et al., 2013). On the other hand, the addition of P sometimes inhibits As uptake by plants (Huang et al., 2007; Wang et al., 2002). As an example, for the hyperaccumulator *Pteris vittata*, growing in hydroponic solution (Tu & Ma, 2003), phosphate can decrease arsenate concentrations in roots and fronds by 23-25 %. This behavior was also observed when cultivated in sand, suggesting that, in these environments, P competes with As in *Pteris vittata*, resulting in less As(V) accumulation in plant tissues at higher phosphate exposure (Huang et al., 2007).

Phosphorus and arsenic uptake by Crambe

Phosphate uptake by plants also can be affected by As in soil. More As in soil resulted in lower P concentration in plant's tissue, as observed for *Crambe abyssinica*. Due to arsenate and phosphate competition in transportation systems and As phytotoxicity effects, P uptake decreased in Crambe with increasing As in soil. This behavior was not observed in *Pteris vittata*, when phosphate uptake was increased by moderate As doses in solution (Tu & Ma, 2003).

Greater arsenic uptake was observed in the P150 and P200 treatments, which suggests that phosphate in *Crambe abyssinica* induces more As uptake. On the other hand, P uptake decreased with soil As addition, suggesting that Crambe uptakes more arsenate instead of phosphate when both of them are present in high concentrations in soil. This behavior may be related to the high affinity uptake system, which synthesis is suppressed as an adaptation to arsenate tolerance. But, in this case, tolerant plants may have problems to uptake phosphate from soil (Meharg & Macnair, 1992).

As previously mentioned, arsenate uptake by plants is related to phosphate transporters. According to Zhao et al. (2009), the phosphate transporters may vary in arsenate affinity. Also, the P transport efficiency is related to the type and quantity of P transporters in plant tissues (Smith et al., 2003). Arsenate uptake decrease in a lower P soil concentration cannot be considered a tolerance mechanism, but an adaptation to nutrient and contaminant balance in soil (Macnair et al., 1992). As reported to Meharg & Macnair (1992), plants generally adapt the phosphate uptake system as a mechanism to reduce arsenate uptake, although, the suppression of this system in tolerant plants can be related to an adaptation to a nutrient-deficient site.

Phosphate did not change *B. juncea* dry matter response when cultivated in As-soil (Grifoni et al., 2014). On the other hand, P addition in soil resulted in biomass increasing of *Pityrogramma calomelanos* growing in soil with As concentrations from 136 to 269 mg kg^{-1} (Jankong et al., 2007).

As observed in this experiment, P application in soil can result in higher As accumulation. The same result was observed in *B. juncea* growing in As contaminated soil, in which P addition increased the bioconcentration factor by 3.4-fold. (Bolan et al., 2013). Phosphate application in As-soil may also decrease phytotoxicity effects caused by As, resulting in more biomass production (Tu & Ma, 2003) and consequently, increase As removal from soil. However, P application can also limits As uptake as observed in *Pteris vittata* (Tu & Ma, 2003).

Crambe removes As from soil

According to Rascio & Navari-Izzo (2011), about 25 % of reported hyperaccumulators plants belong to Brassicaceae family. In this experiment, Crambe produced high biomass and accumulated high concentration of As in shoots (up to 21 mg/pot and 759 mg kg^{-1} of As in As150/P200 treatment). Similar results were reported by Artus (2006), that compared crambe with other brassicas in hydroponic solution, and found As concentration of 270 mg kg^{-1} in shoots of Crambe cultivated in 70 μmol L^{-1} of sodium arsenate and 250 μmol L^{-1} of phosphate. Crambe accumulates 21-fold more As in shoots than *B. juncea* when treated with 70 µmol L^{-1} of sodium arsenate and 250 µmol L^{-1} of phosphate (Artus, 2006), showing that this plant has an efficient root-to-shoot transport system. Other results suggests *B. juncea* has low BF and TF (less than 1), even after P addition in soil (Grifoni et al., 2014), corroborating the results found by Artus (2006).

Even though Crambe cannot remove the same quantity of As from soil as *Pteris vittata* does, this plant has a good potential for As extraction. Crambe is well adapted to northern climates (Artus, 2006), and also produces greater biomass than other hyperaccumulators plants. According to the best result of this experiment for As content in shoots (Fig. 3) of Crambe (As100 and P200), and assuming a plant density of 120 plants m^{-2}, *Crambe abyssinica* could potentially remove up to 9 kg ha^{-1} of As from soil and accumulate it in shoots along its life cycle (approximately 90 days). High biomass and As concentrations in that biomass are characteristics that define the ability of the hyperaccumulator plant to remove As from soil (Tu & Ma, 2002).

An arsenic hyperaccumulator plant should have translocation and bioconcentration factors greater than one (Ma et al., 2001; Zhang et al., 2002). *Crambe abyssinica* TF and BF reached 5 and 61, respectively. Arsenic concentration in aboveground biomass reached maximum of 759 mg kg^{-1}, which represents less than 10 % of As concentration in aboveground of *Pteris vittata* growing in similar conditions (Tu & Ma, 2003). *Pteris vittata* is known as the As hyperaccumulator that removes the greater amounts of As from soil (Ma et al., 2001). The experiments showed that As concentrations in *Pteris vittata* shoots can vary from 4 to 25 times greater than in roots (Tu & Ma, 2002). However, even though *P. vittata* translocates more As than *Crambe abyssinica*, both of them had the TF decreased with As increasing in soils, due to a decrease in As in shoots (mainly in Crambe leaves) and an increase in roots (Tu & Ma, 2002).

Crambe seeds have less arsenic than other tissues

Crambe limits As translocation to seeds as an adaptation to guarantee seeds dissemination. Arsenic concentration in grains was less than 2 % of the total As in plant, which means that this plant avoid As translocation to seeds, keeping it on leaves and stems.

Phosphorous concentration per mass of seeds decreased with As in soil. Similar behavior was observed for As content in shoots. A decrease in P content may be related to plant uptake limitations and less biomass production, due to As phytotoxicity. For high As treatments, less roots per plant were observed than other treatments, resulting in an uptake limitation of both nutrients and As.

In the same way that plants have limited As translocation to seeds, germination rates were not affected by As in soil, except for P150 treatment (Fig. 5). Crambe seeds from As-treated soils showed high germination rates despite As phytotoxicity to the plant, and this variable correlated positively with As concentration in leaves, stems, and roots. These results suggest that Crambe developed adaptations to avoid As translocation to seeds, such as accumulation of this element in stems or leaves, as mentioned before.

Conclusions

In my experiments, As uptake by crambe increased as soil phosphate additions increased. This plant can be considered a hyperaccumulator plant due to the higher translocation and bioconcentration factors. Crambe also produces seeds with greater germination rates even after being cultivated in As contaminated soils.

Based on my research, crambe is a potential plant species for As phytoremediation programs, due to its capacities grow robustly in As contaminated soils, take up arsenic from the soil, translocate the As to aboveground biomass and store few amounts of As in seeds.

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CHAPTER 2: Spatial distribution and speciation of arsenic in the rhizosphere and tissue of *Crambe abyssinica* grown in an arsenic-treated soil

Abstract

Phytoremediation is one of the techniques used to remove arsenic from contaminated soils, avoiding contamination of food and water. In evaluating *Crambe abyssinica* as a potential plant for phytoremediation, the objective of this research was to map the spatial distribution and speciation of As in As(III) and As(V)-treated soil, the rhizosphere, and plant parts of Crambe. Plants were cultivated in a greenhouse for 20 days. To visualize root during the experiment, plants were cultivated in chambers designed for the roots to grow between two acrylic plates separated by 5 mm. A sandy Oxisol from Brazil was used in the experiments. The soil received 10.7 mM solution of As(III) or As(V), composing two different treatments. Arsenic spatial distributions were mapped in intact soil-rhizosphere-root samples and separately in Crambe leaves on the XRF beamline at LNLS. The μ-SXRF analyses were performed using a whitebeam. After mapping, selected points (hotspots) with higher As fluorescence intensity were identified, and μ-XANES analyses were performed using monochromatic X-rays to identify As species in the root-rhizosphere-soil continuum. After data collection, plants were harvest, separated into leaves and roots for trifluoracetic acid (TFA) extraction and speciation by HPLC-AFS. Our results showed that As is located mainly in veins of *Crambe abyssinica* leaves, and most of the As in leaves is arsenite. In the root-rhizosphere system, differences between arsenic species in soil and rhizosphere were found for both samples suggesting that Crambe roots change the rhizosphere in ways that induces changes in As speciation by arsenate reduction or promoting methylation in order to detoxify arsenic.

Introduction

Arsenic (As) is naturally present in soils, rocks, and water, causing many diseases related to human exposure such as cancer and mental issues. For this reason, As is considered one of the most hazardous elements for human health. In order to decrease arsenic exposure to animals and humans, techniques for As immobilization or removal from soils were developed. The most common technique is phytoremediation, due to its low cost and low demand for technology (Kabata-Pendias, 2011).

The success of phytoremediation depends on a plant's tolerance and biomass production. The two types of plants used in this technique are tolerant and hyperaccumulator species. Tolerant plants grow in As-contaminated soils but do not translocate As to shoots. These plants can immobilize the contaminant into the roots to avoid its uptake. On the other hand, hyperaccumulators can uptake and translocate arsenic to shoots, facilitating removal of the contaminant from soil (McGrath et al., 2002). The success of phytoremediation also depends on contaminant dynamics in plants. Arsenic uptake is related to both the bioavailability of chemical species in the soil solution and a plant's ability to take up different As species from roots to shoots, which depends on plant type (Rajkumar et al., 2012).

The most common arsenic oxidation state taken up by plants is As(V), e.g., as arsenate oxyanion, which is dominant in aerobic soils. Terrestrial plants are able to take up arsenate and detoxify it by reducing As(V) to As(III). Subsequently, complexes with thiol-reactive peptides are formed to avoid damage to plant cells (Larios et al., 2012; Paulose et al., 2010; Zhang et al., 2002). In reducing environments, such as paddy soils, the dominant As oxidation state is arsenite, As(III). Aquatic plants absorb arsenite via aquaporin channels that transport water and neutral molecules such as silicic acid and arsenious acid (Seyfferth et al., 2010). In rice, for example, arsenic uptake occurs due to influx (Lsi1) and efflux (Lsi2) transporters for silicic acid (Ma et al., 2008). Methylated As species, including monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA), are also found in soil. However, they usually occur in lower proportion than inorganic species, and plant uptake mechanisms of methylated species are still unclear (Zhao et al., 2009).

Some plants from the Brassicaceae family are known as potential species for arsenic phytoremediation, for example, *Brassica juncea* and *Crambe abyssinica* (Paulose et al., 2010; Artus, 2006; Pickering, 2000). Experiments using hydroponic solutions (Artus, 2006) have showed that *Crambe abyssinica* is arsenic tolerant and suggested that this plant is suitable for phytoremediation. Crambe grows in medium-low temperature environments and produces 1000-1500 kg ha^{-1} of oilseeds that are commonly used in the lubricant and cosmetic industries.

The potential utility of using crambe for As phytoremediation is already recognized; however, As concentrations and species taken up by this plant when cultivated in soil are still not known. In order to better comprehend distribution of As species in crambe, it is important to study the rhizosphere, which is the plant-soil interface where chemical and biological processes can change arsenic speciation that might affect plant uptake (Kidd et al., 2009; Zhao, et al., 2009). These changes are still not well known due to limitations in defining the rhizosphere and identifying As speciation. Despite being dominant in soils, arsenate can be converted to arsenite in the rhizosphere as a plant detoxification mechanism. Moreover, anaerobic sites in rhizospheres create a suitable environment for As(III) persistence (Yang et al., 2012).

This work aimed to determine whether *Crambe abyssinica* changes As speciation in the rhizosphere as a mechanism that potentially affects As uptake, and to evaluate any co-localization of As with other elements in Crambe leaves as a mechanism that facilitates As accumulation.

Material and Methods

Plant Growth

Two 100 cm^3 portions of a sandy Oxysol were spiked with 10.7 mmol L^{-1} solution of As(III) and As(V) (As_2O_3 and $Na_2HAsO_4.7H_2O$, respectively), then incubated for 34 days. The arsenic added to each soil sample was 0.2 mg of As cm^{-3}.

To create a system for visualizing root growth, transparent acrylic plates were configured to hold soil. Each system was composed of nine 50 x 80 mm transparent acrylic plates bonded laterally with double-sided tape. The plates were filled with a 2-mm thickness of As-contaminated soil [one with As(III) and other with As(V)]. Three *Crambe abyssinica* seedlings, pre-germinated for 12 days, were placed in each treated system. Recommended rates of macro- and micro-nutrients were added before planting (Alvarez V, 1974). In order to adjust the soil pH to 6.0, $CaCO_3$ and $MgCO_3$ (4:1 ratio) were mixed with the bulk soil. Phosphorous was added as $NH_4H_2PO_4$ to obtain 200 mg dm^{-3} de P in soil. Additions of KCl and NaCl were used to maintain equivalent K and Na additions to each treated soil. Plants were grown for 20 days in a greenhouse and watered once a day using a sprinkler containing deionized water.

Figure 1. Transparent acrylic plates designed to create a system for visualizing root growth.

Micro X-ray Analyses

After the 20-day growth period, the whole intact plant-soil system was transported to the Brazilian Synchrotron Light Laboratory (LNLS) for synchrotron X-ray analyses. Samples were prepared in an acrylic glovebox, under N_2 atmosphere. For leaf analysis, fresh leaves were cut from the plant, covered with Ultralene® film and fixed on a sample holder. For root-soil samples, the pots were cut to get the visibly best section of root and soil. The front acrylic plate of this section was exchanged with an acrylic plate of equal dimensions, but with a

20 x 30 -mm window covered by Ultralene® film. All soil that extruded outside of the window was removed. These plates were joined and placed on the XRF beamline sample stage for analysis.

Synchrotron micro X-Ray Fluorescence (μ-SXRF) and micro X-ray absorption near-edge spectroscopy (μ-XANES) data were collected. At first, soil-rhizosphere and leaves samples were imaged on 10.0 x 8.0 mm and 7.0 x 6.5 mm area, respectively, with a white beam of 30 μm x 30 μm spatial resolution and a dwell time of 200 ms per pixel, while continuously flushing the sample with N_2 gas. μ-XRF images were processed using the PyMCA® software (Solé et al., 2007).

After all sample were mapped for As, hotspots along the root-soil samples with maximum fluorescence intensity for As were identified in three different sample areas: root, rhizosphere, and soil. The white beam was replaced by a monochromatic beam to obtain arsenic μ-XANES spectra. At least 6 scans were collected on each spot between -80 and 133 eV relative to the edge energy set at 11867 eV (arsenic K-edge energy). Step sizes and dwell times were 2.0 eV and 4 s in the pre-edge and post-edge regions (-80 to -20 eV and 100 to 133 eV), and 0.5 eV and 8 s across the near-edge region (-20 eV to 100 eV).

Arsenic K-edge μ-XANES data processing and linear-combination fitting (LCF) analyses were performed using the ATHENA® software (Ravel & Newville, 2005). Multiple scans were merged and smoothed with a Boxcar algorithm with Kernel size 5 to decrease spectral noise. The μ-XANES sample spectra were all energy shifted by 0.2135 eV based on the average alignment of a μ-XANES spectrum for As_2O_3 and MMA standards collected along with samples, and previous calibrations of these standards to a known reference energy on SSRL Beamline 2-3. The Si(111) monochromator energy calibration was assumed to be constant throughout the data collection period.

The μ-XANES spectra collected from crambe samples and fitting standards were baseline corrected across an energy range from -65 to -30 eV (relative energy) using a linear regression and, normalized using a linear regression across the energy range 10 to 115 eV relative to the edge energy (E0) set to the first derivative maximum for each spectrum (Kelly et al., 2008). The LCF analyses (Kelly et al., 2008) of the normalized spectrum from each sample was performed across an energy range of -15 to 35 eV relative to E0, using Manceau approach standard elimination (Manceau and Drifts, 1993).

Chemical Speciation by Extraction

After data collection in the synchrotron facilities, leaves and roots were separated, washed thoroughly with tap water and, then rinsed with a 0.01 mol L^{-1} solution of HNO_3 followed by three rinses with deionized water. Subsequently, samples were stored at - 20 ºC until analysis.

Samples were ground under liquid nitrogen and an N_2 atmosphere in a glove bag (Atmosbag®, Sigma-Aldrich). A subsample of approximately 0.10 g, wet weight (WW) was placed in a digestion tube, and 2.0 mL of 2 mol L^{-1} solution of trifluoracetic acid (TFA, Sigma-Aldrich®) were added. The digestion tubes were heated in a digestion block at 100 ºC for 6 h. Subsequently, the extract was evaporated to dryness, and the residue material was dissolved with deionized water, filtered through a 0.22 μm membrane, and the filtrate was diluted to 25 mL. These extracts were stored at -20 ºC until analysis by High Performance Liquid Chromatography coupled to Atomic Fluorescence Spectroscopy (HPLC-AFS) to measure arsenate [As(V)), arsenite (As(III)], monomethyl arsenic (MMA) and dimethyl arsenic (DMA).

Results

Soil-rhizosphere SXRF maps

Figure 2 shows SXRF maps of As, P, K, Ca, Fe, and Si around roots of Crambe grown in soil samples treated with either As(III) or As(V), along with locations where As K-edge μ-XANES spectra were collected (discussed below).

The roots are well defined in the maps of As, K, and Ca for both treatments. However, the distribution of As is more diffuse than K in the near-root region, which we consider to be the rhizosphere in regard to samples for XANES analysis.

The greater fluorescence signal for As in a diffuse zone around the roots, suggests an accumulation of As in the rhizosphere relative to the bulk soil.

The fluorescence signals of P, Fe, and Si showed the minimal contrast between the roots and bulk or rhizosphere soils, indicating a lack of accumulation of these elements around the root relative to the bulk soil. Generally, the SXRF signal intensities were similar for the same element between the two As treatments.

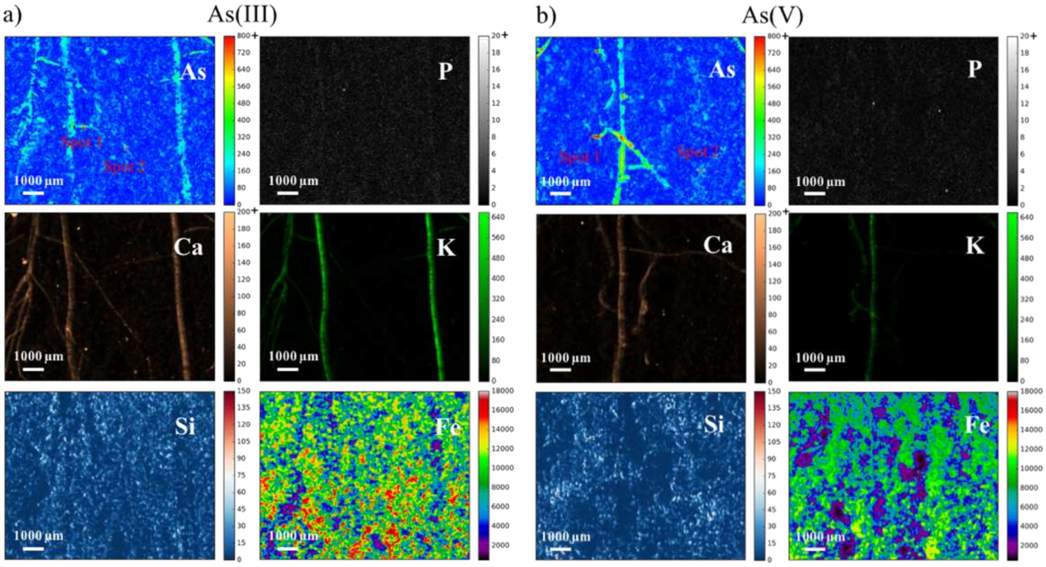

Figure 2. SXRF images (10 mm x 8 mm) of various elements in the soil-root-rhizosphere region of Crambe grown in soil treated with 200 mg As dm^{-3} as either (a) As(III) or (b) As(V). The color intensity scales for each element are the same for corresponding maps: 0-1377 cps (As), 0-31 cps (P), 0-660 cps (K), 0-1106 cps (Ca), 500-18045 cps (Fe), and 0-153 cps (Si).

Soil rhizosphere XANES

Figure 3 shows micro-XANES spectra for As in the bulk soil and rhizosphere soil for each of the As treatments, along with standards that were included in the best-fits to the sample spectra using LCF analysis (Table 1). Spectra taken directly on roots were not considered reliable because the X-ray beam penetrates the root and includes signals from soil material behind the root. Only standards of As(V) or As(III) adsorbed on boehmite [As(V)-boehmite and As(III)-boehmite, respectively] and sodium monomethyl arsenate [MMA(V)] were needed to fit XANES spectra for all four soil samples. The R-factors (residuals) of fits ranged from 0.002 to 0.004, and the sum of weighting factors ranged from 1.00 to 1.03 and was renormalized to sum to 1.00 (Table 1).

All the four samples could be fit well with As(V)-boehmite (70-100 %), balanced by As(III)-boehmite and MMA(V), in As(III)- and As(V)-treated samples, respectively (Table 1). However, differences in the micro-XANES spectra and fitting results between rhizosphere soil and bulk soil samples, suggest that Crambe roots induce changes on As speciation. Specifically, the rhizosphere of the As(III) treatment contains more As(III) than the respective bulk soil, as indicated by a shoulder on the low-energy side of the white line (Fig. 3) and a greater proportion of As(III)-boehmite standard in the fits (Table 1). Also, the As(V)-treated sample suggested the presence of more MMA(V) in the rhizosphere soil than in bulk soil, as indicated by a shift of the white line to a lower energy (11871 eV) related to the bulk soil (11872 eV) spectra, and a greater portion of MMA(V) standard in the fits (Table 1). Based on the greater proportion of MMA(V) in the fit of single micro-XANES spectra of rhizosphere soil relative to the bulk soil for the As(V)-treated sample, we hypothesize that Crambe's root has induced the formation of organic arsenic in the rhizosphere for this treatment. For the As(III)-treated soil, the root appeared to reduce As(V) formed in the soil back to As(III), or inhibit oxidation of As(III) to As(V) initially. No MMA(V) was included in the best fit of the As(III)-treated samples.

Table 1. XANES fitting results

Spot #	Sample description	As(V)-boehmite (%)	As(III)-boehmite (%)	MMA(V) (%)	R-factor
1	As(III)-rhizosphere	67 ± 1	33 ± 1		0.002
2	As(III)-soil	100 ± 0			0.004
3	As(V)-rhizosphere	69 ± 2		31 ± 3	0.003
4	As(V)-soil	91 ± 2		9 ± 2	0.003

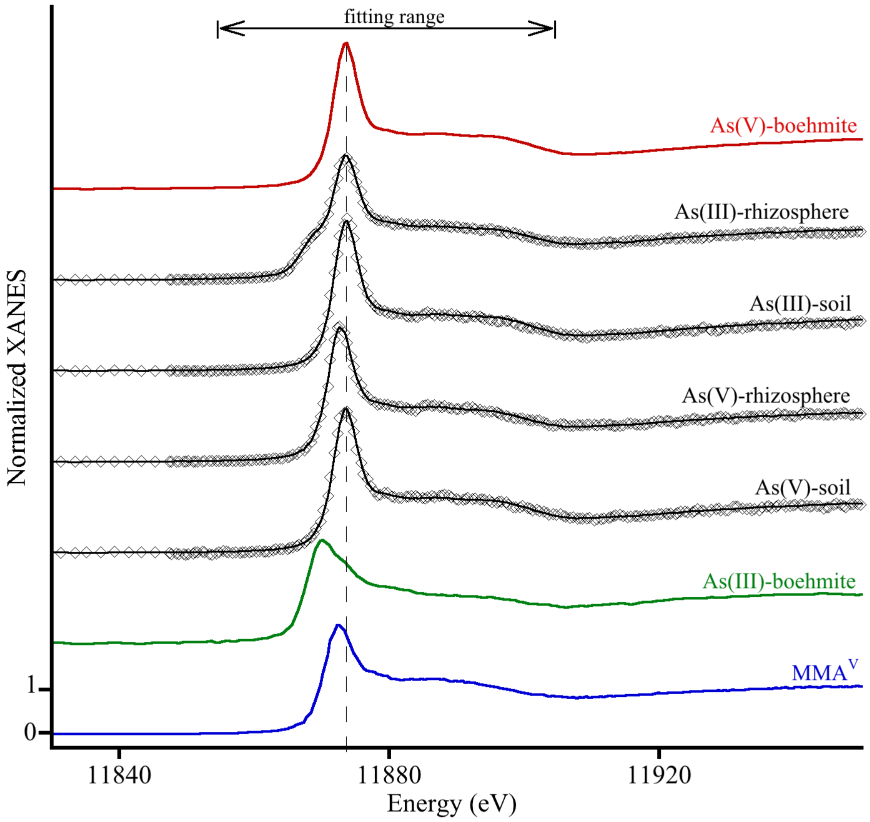

Figure 3. Arsenic K-edge XANES spectra for bulk soil and rhizosphere soil of Crambe grown in Oxisol material treated with either As(III) or As(V), along with spectra for standards that were included in the best-fit models of the samples.

Arsenic in Above-Ground Plant Parts

SXRF maps of As, P, K, Ca, Fe, and Mn in *Crambe abyssinica* leaves are shown in Fig. 4. In maps of leaves from both As(III)- and As(V) treatments, we observed higher As signal in veins. Phosphorus and K are widely distributed around the whole mapped area in both treatments, whereas Fe fluorescence signal is very weak.

In leaves from the As(III) treatment, Ca appears to be associated with trichomes, along with Mn at the bases of the trichomes. However, no co-localization between these elements and As was observed in Crambe leaves. Although the roots from the As(V) treatment had high fluorescence signals for Ca (Fig. 2), the leaves of this plant showed no trichomes nor co-localization of Ca and Mn.

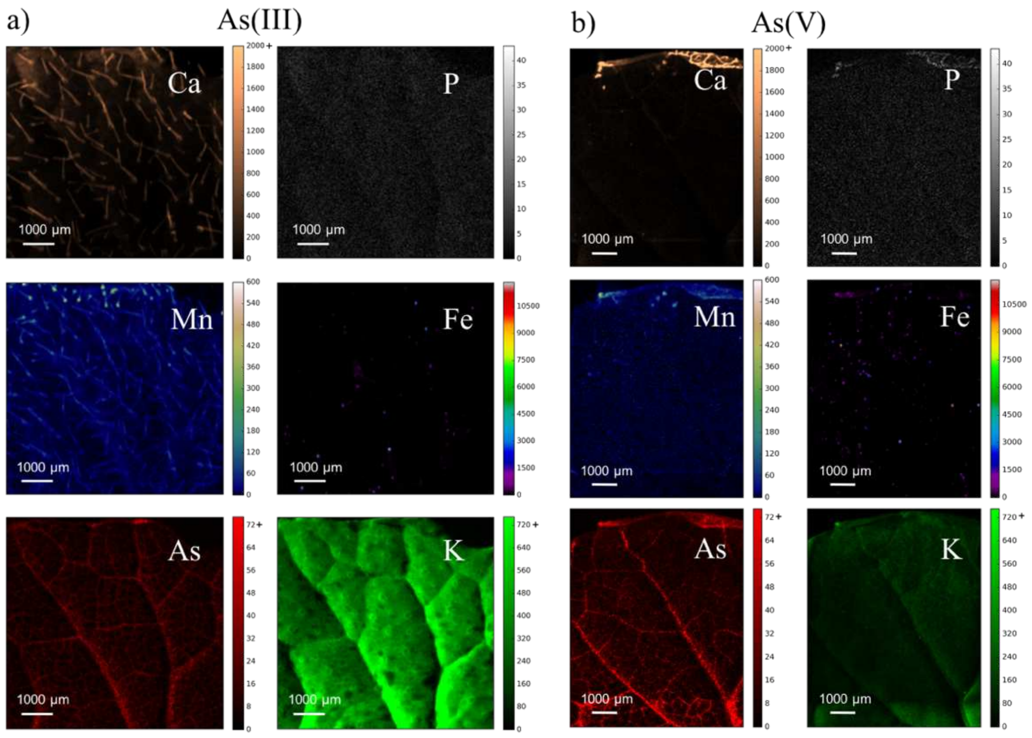

Figure 4. SXRF images of various elements in the leaves of *Crambe abyssinica* grown in soil treated with 200 mg As dm^{-3} as either (a) As(III) (7 x 6.5 mm) or (b) As(V) (7 x 9 mm). The color intensity scales for each element are the same for corresponding maps: 0-8300 cps (Ca), 0-43 cps (P), 0-600 cps (Mn), 0-11850 cps (Fe), 0-320 cps (As), and 0-1375 cps (K).

Chemical As speciation in *Crambe abyssinica*

Figure 5 shows As species concentration in leaves and roots of *Crambe abyssinica* in response to the As treatments. Arsenite concentrations in leaves were 19.18 µg g^{-1} and 41.03 µg g^{-1} for As(III)- and As(V)-treated plants, respectively. On the other hand, arsenate concentrations were 11.00 µg g^{-1} and 11.42 µg g^{-1} for leaves from the As(III)- and As(V) treatments, respectively, which indicates no difference in As(V) translocation to leaves between the treatments. The As(III)-treated plants had translocated 30.18 µg g^{-1} of As to leaves, whereas those treated with As(V) had translocated 53.07 µg g^{-1} of As. Arsenite was the As species more present in leaves, regardless the As-treatment.

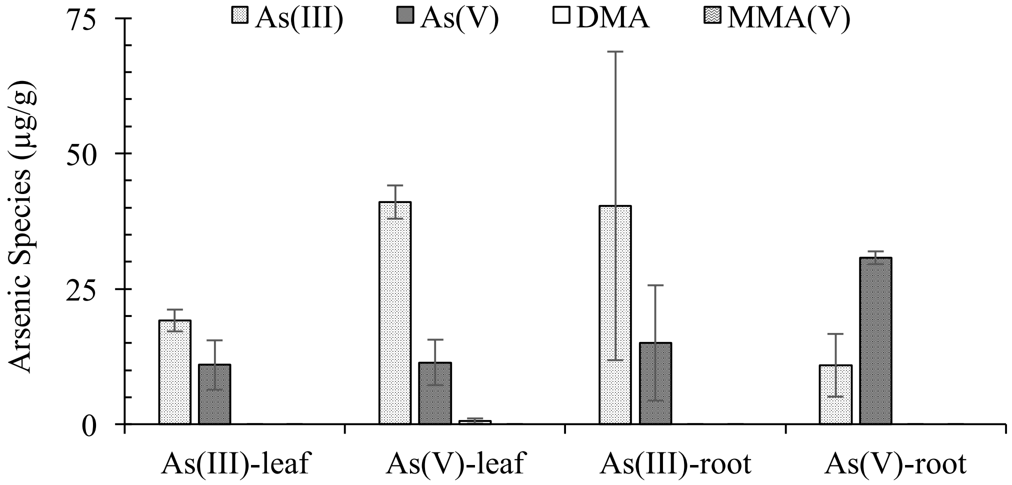

Figure 5. Arsenic speciation of As(III), As(V), DMA, and MMA(V) extracted by TFA from *Crambe abyssinica* leaves and roots, for As(III) and As(V) treated soils. Bars represent standard errors. n = 2.

The As(V)-root samples had more arsenate (30.78 µg g^{-1}) than arsenite (10.93 µg g^{-1}), whereas the As(III)-root had a greater arsenite concentration (40.32 µg g^{-1}) relative to arsenate (15.04 µg g^{-1}).

No As-methylated species were found in this experiment, except that the leaf from the As(V) treatment that showed 0.62 µg g^{-1} of DMA, representing only 1.17 % of total As in the samples. This minor level might be an artifact of the overlap of chromatography peaks between As(III) and DMA for this sample containing higher As(III) concentration.

Discussion

Soil-rhizosphere-root SXRF maps

The prominent As signal observed in a diffuse zone around crambe roots in soil-rhizosphere-root maps suggests higher As concentrations in the rhizosphere relative to bulk soil. The same difference has been reported in rhizosphere of *Pteris vittata* growing in gold mining regions (Acosta, et al. 2015 and Gonzaga et al., 2009).

Higher As concentration in rhizosphere and its positive correlation to soil solution pH and dissolved organic carbon, are suggested to be results of the root exudates capacity to acidify rhizosphere soil in order to release As to plants (Gonzaga et al., 2009). However, this capacity is also associated with tolerance and uptake plants for As, since some plants inhibit As uptake in roots, avoiding its translocation to above ground parts.

Soil-rhizosphere XANES

The presence of As(V) in As(III)-treated soil is potentially related to the instability of arsenite in dried soils. Due to the pH and high redox potential of aerobic soils, arsenite applied to soil can be oxidized to arsenate (Kabata-Pendias, 2011). On the other hand, the differences between soil and rhizosphere XANES spectra and results from LCF analysis, corroborate the known changes made by root exudates of As-tolerant plants in order to modify As species, generally from As(V) to As(III) or to methylated species, before uptake (Gonzaga et al., 2009; Kidd et al., 2009).

Arsenic oxidation changes in As(III)-rhizosphere suggest that As can be reduced by root exudates before plant uptake, which is a common mechanism in As hyperaccumulator plants such as *Pteris vittata* (Mathews et al., 2010; Xu et al., 2014) or, that root exudates can inhibit oxidation of applied As(III). These changes in the rhizosphere are mechanisms to detoxify As(V) by reduction to As(III), which is less toxic for tolerate plants.

Leaf SXRF maps

According to Werner et al. (2016), crambe's vascular system is formed mostly by a single collateral vascular bundle of small size that is present across the whole mesophyll. For this reason, maps of leaves from As(III) or As(V)-treatments showed As spread around the whole mapped area, with higher signal in veins. It is not possible to affirm that more As is concentrated in crambe veins, because samples were prepared *in vivo*, without cuts made in microtome. In this case, thickness and density of the plant material affect X-ray absorption.

Leaf samples from the As(III) treatment showed Ca-rich trichomes in the whole area mapped. These structures were also found by Leigh Broadhurst et al. (2009) in Alyssum plants, suggesting that calcium carbonate and/or Ca-oxalate crystallites are the principal components of trichomes in some plant groups. These authors also found expressive concentrations of Mn in trichomes bases, as we observed in the leaf from the As(III) treatment (Fig. 4).

Arsenic species in leaves and roots

Based on TFA extraction results, Crambe leaves had more As(III) than As(V) even in As(V) treatments. Arsenic species predominantly present in plants grown in As-contaminated sites is As(III), regardless of the inorganic form [As(III) or As(V)] in the soil (Zhao et al., 2009). For Crambe roots, the results showed that As speciation depends on the oxidation state of As applied to the soil, although As(III) was present for both of the treatments. The predominance of As(III) in Crambe leaves in both As(III) or As(V) treatments indicates that between the soil and the leaves, As(V) is reduced to As(III), at least for the As(V) treatment, which is a previously reported plant-tolerance mechanism (Smith et al., 2007). For the As(III) treatment, the As(III) may have been taken up directly from soil. When exposed to As(V), plants reduce As(V) to As(III), then, arsenite is detoxified by thiol-reactive peptides complexes formation such as γ-glutamylcysteine, glutathione, and phytochelatins (Paulose et al., 2010; Pickering et al., 2000).

A predominance of arsenite in plant tissues has been reported in other arsenic speciation studies. Arsenite is also the majority species found in tissues of *Pteris vittata* (Mathews et al., 2010), *Pityrogramma calomelanos* (Francesconi et al., 2002), *Raphanus sativus* (Smith et al., 2007), and *Brassica juncea* (Pickering et al., 2000). Pickering et al. (2000) suggest that the As(V) is reduced to As(III) in the plant, and makes sulfur bonds forming As(III)-tris-glutathione complex. *Crambe abyssinica* belongs to the same family of *Brassica juncea,* therefore it is expected that the same mechanism is adopted by Crambe in order to uptake and tolerate high concentrations of arsenic.

In plants, As(V) is suggested to be taken up by phosphate transport mechanisms and promptly reduced to As(III) or transported via xylem and stored in high quantities in cell vacuoles (Gerényi et al., 2016). In *Brassica juncea*, after root uptake, a small fraction of As(III) and As(V) is exported by xylem to shoots (Pickering et al., 2000). This might be the reason that we found both As(V) and As(III) in Crambe leaves, regardless of treatment.

Despite changes in As speciation in the rhizosphere of the As(V)-treated soil, no methylated arsenic species were found in Crambe roots and leaves [except for As(V)-leaf]. This suggests that Crambe is not able to methylate As species before uptake and transport or, if methylation occurs, the organic species are translocated in small quantities compared with inorganic arsenic as suggested by Smith et al. (2007). Abedin et al. (2002) also reported from short term rice studies that translocation of MMA and DMA was considerably less than that of As(III) or As(V).

The potential of *Crambe abyssinica* to grow in As contaminated sites is related to its high capacity to detoxify As before uptake. For this reason, the plant makes changes in rhizosphere, reducing arsenate to arsenite, or by As methylation, as suggested by our XANES results. On the other hand, the lack of methylated species in Crambe leaves and roots as shown by extraction chemical speciation results, suggests that the As(V) reduction in rhizosphere may be the most common mechanism adopted by *Crambe abyssinica* for arsenic tolerance.

Conclusion

My results showed that Crambe roots change the rhizosphere of an Oxisol in ways that induce changes in As speciation, specifically arsenate reduction to arsenite and As methylation. We found no co-localization of As with other inorganic elements that we measured in the leaves and rhizosphere of *Crambe abyssinica.* Arsenic is distributed in veins of *Crambe abyssinica* leaves and the arsenite was the predominant As species in the plant structures, regardless of the As species in soil.

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GENERAL CONCLUSIONS

Although the high translocation and bioconcentration factors (5.09 and 61.11, respectively) suggest *Crambe abyssinica* as arsenic hyperaccumulator plant, the correct P fertilization is an important factor to improve phytoremediation results. Generally, the more soil-applied phosphate (200 mg P dm^{-3} soil being the maximum application evaluated here) the more As uptake was enhanced and the greater the shoot biomass production, resulting in up to 21 mg/pot of As extraction from soil.

Crambe abyssinica translocated a maximum of 759 mg kg^{-1} of As to aboveground parts. Furthermore, the best treatment for increased As content in shoots (As100/P200) suggests that Crambe can remove up to 9 kg ha^{-1} of As from soil. Seeds produced from plants grown in As-contaminated soils had germination rates up to 60 and 96 %, and contained less than 2 % of total As in the whole plant. These results suggest that this plant has potential for use in As phytoextraction from contaminated soil.

Crambe showed similar behavior to other As tolerant plants, which change As speciation in rhizosphere in order to translocate it to shoots. The most common As species in aboveground parts was As(III), such as in other plants. In Crambe leaves, the arsenic seems to be concentrated in veins, due to their wide distribution around the whole mesophyll.

Our results, associated with the short life cycle of Crambe, suggest that *Crambe abyssinica* has potential for fast and efficient phytoremediation of As contaminated soils.