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# Using response surface methodology to assess the effects of iron and spent mushroom substrate on arsenic phytotoxicity in lettuce (*Lactuca sativa* L.)

Namin Koo[a], Hun-Je Jo[b], Sang-Hwan Lee[c], Jeong-Gyu Kim[a,*]

[a] *Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Republic of Korea*
[b] *Water Environment Center, Korea Institute of Science and Technology, Seoul 130-650, Republic of Korea*
[c] *Technology Research Center, Mine Reclamation Corporation, Seoul 110-727, Republic of Korea*

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ABSTRACT

The effects of iron (Fe) and spent mushroom substrate (SMS) arsenic (As) phytotoxicity towards lettuce in artificial soils were investigated to separate the adverse soil parameters relating to As toxicity using a response surface methodology. SMS induced the root elongation of lettuce in both control and As-treated soils. However, in phytotoxicity test using a median effective concentration ($EC_{50}$) of As, Fe and the interaction between both parameters (Fe*SMS) significantly affected $EC_{50}$, which explained 71% and 23% of the response, respectively. The refined model was as follows: $EC_{50}$ of As ($\mathrm{mg\,kg^{-1}}$) = 10.99 + 60.03 × Fe – 10.50 × Fe*SMS. The results confirmed that the soil parameters relating to the As mobility in soils were important factors affecting its toxicity. In conclusion, Fe significantly reduced the As phytotoxicity. However, although SMS enhanced the root elongation, SMS in As-treated soils decreased $EC_{50}$ of As on the root growth via its interaction with Fe. Despite the limitations of the artificial soils and range of parameters studied, the application of this statistical tool can be considered a powerful and efficient technique for interpretation and prediction of the complicated results caused by the interactions between many factors within the soil environments.

## 1. Introduction

Arsenic (As) is a ubiquitous trace element in the environment and it is commonly recognized as being toxic to animals, plants and humans [1,2]. Therefore, to remediate As-contaminated sites, a variety of physical, chemical and biological approaches have been applied. Of these, the application of various soil amendments into As-contaminated soils has been recognized as a cost-effective and environment-friendly technique [3]. The chemical stabilization of As, using soil amendments, has been considered for the remediation of As-contaminated soils, with the possible amendments for reducing the As mobility and improving the soil quality having been reported in many investigations [4,5].

The iron (Fe)-rich amendments are representative additives for the As immobilization, and have been utilized more than any other inorganic amendment [6]. Since the surface of Fe oxide has adsorptive potential for dissolved As in soil solutions [7], it would be very effective for the As stabilization via its adsorption onto the surface of Fe oxide. Hartley et al. [8] conducted a study on the reduction in the As mobility in landfill site soils using various Fe amendments. They reported that the water-extractable As in Fe-amended soil was reduced from 459 to 40 $\mu\mathrm{g\,kg^{-1}}$. Gemeinhardt et al. [9] reported that up to 90% of leachable As in the Fe-amended soil was immobilized via a column test. Also, the addition of Fe-halide salts to contaminated soils decreased the bioaccessibility of As in a physiologically based extraction test [5].

In addition, organic matter (OM)-rich materials, including agricultural by-products and wastes, have been proposed and tested as additives for the remediation of contaminated soils [6,10]. Organic amendments significantly reduced the As toxicity in the amended chromated-copper-arsenate (CCA)-contaminated soils via the adsorption of As by the organic matter fraction [11]. Heeraman et al. [12] demonstrated that the growth of *Vulpia myuros* L. in compost (yard trimmings) amended As-contaminated soils significantly increased, despite increases in labile As. Juwarkar et al. [13] also reported on the growth of *Jatropha curcas* in As-contaminated soils applied with the biosludge, and showed greater enhancement to that of the control. However, studies on the stabilization and phytotoxicity of As in soils using organic amendments have also given rise to controversial results. Some reports have shown that the incorporation of organic amendments increased the As mobility due to induction of dissolved organic carbon (DOC) concentrations [14,15] and pH values [16,17]. Hence, remediation with organic amendments could be simultaneously performed to enhance the As mobility and its extraction with As hyperaccumulators, such as *fern* species [15].

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* Corresponding author. Tel.: +82 2 3290 3024; fax: +82 2 921 7628.
*E-mail address:* lemon.jg.kim@gmail.com (J.-G. Kim).

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**Table 1**
Experimental set-up used in the central composite design (CCD) and the effects of iron (Fe) and spent mushroom substrate (SMS) on germination and root elongation of lettuce (*Lactuca sativa* L.) seeds.

| Experimental point | Treatment | Coded levels | | Actual levels (%) | | Ger[a] (%) | RE[b] (mm Petri-dish$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | Fe | SMS | Fe | SMS | | |
| Cube | 1 | −1 | −1 | 1.00 | 1.00 | 197 ± 3 | 133 ± 26 |
| | 2 | −1 | −1 | 1.00 | 3.00 | 100 ± 0 | 209 ± 22 |
| | 3 | 1 | −1 | 3.00 | 1.00 | 100 ± 0 | 146 ± 16 |
| | 4 | 1 | −1 | 3.00 | 3.00 | 100 ± 0 | 252 ± 9 |
| Star | 5 | 0 | −1.41 | 2.00 | 0.59 | 100 ± 0 | 172 ± 5 |
| | 6 | 0 | -1.41 | 2.00 | 3.41 | 100 ± 0 | 266 ± 28 |
| | 7 | −1.41 | 0 | 0.59 | 2.00 | 193 ± 3 | 193 ± 6 |
| | 8 | 1.41 | 0 | 3.41 | 2.00 | 100 ± 0 | 212 ± 8 |
| Central | 9 | 0 | 0 | 2.00 | 2.00 | 100 ± 0 | 222 ± 14 |
| | 10 | 0 | 0 | 2.00 | 2.00 | 193 ± 7 | 186 ± 11 |
| | 11 | 0 | 0 | 2.00 | 2.00 | 100 ± 0 | 220 ± 6 |

[a] Germination percentage of lettuce seeds.
[b] Root elongation of germinated lettuce seeds in the non-As treated soils.

There were also many reports investigating the effects of co-application of both Fe and OM on the As stabilization in contaminated soils. Bleeker et al. [18] reported that the growth of *Holcus lanatus* in As-contaminated mine spoils treated simultaneously with steel shots and OM largely enhanced, despite slight increases in water-soluble As. However, Mench et al. [16] found that the root weights of maize and dwarf bean in mine soils was completely reduced in treatments amended with both compost and Fe-rich materials (maghemite and magnetite).

While there has been significant development in the understanding of the mobility and phytotoxicity of As in soils treated with Fe- and/or OM-rich materials, the well-defined study on identifying the individual and interactive effects of Fe and OM in the co-addition of those on phytotoxicity of As in soils are still limited. In addition, investigations on the soil parameters relating to the reduction in the toxicity of trace elements in soils, using the response surface methodology, have been limited to a few elements (Cd and Zn), but not As [19–21].

Thus, the objectives of this study were to evaluate the direct effects of the Fe and spent mushroom substrate (SMS) on the root elongation in lettuce (*Lactuca sativa* L.), and the effects of both soil parameters in reducing the As phytotoxicity (using $EC_{50}$) in artificial test soils using the response surface methodology.

## 2. Materials and methods

### 2.1. Experimental design and set-up

The response surface model consists of a group of empirical methodologies for analyzing the relationships between a cluster of controlled experimental parameters (Fe and SMS in the present study) and the observed responses (the root elongation of lettuce in study using non-As treated soil and $EC_{50}$ of As in the phytotoxicity study) according to one or more selected criteria [22]. In the present study, the second-order central composite design (CCD) was adopted to evaluate the effects of the Fe and SMS contents on the root growth of lettuce, and phytotoxicity on exposure to As. The values of parameters selected for CCD are summarized in Table 1. The studied model (3 experimental points and 11 experiments) required for two factors and five levels in the CCD, which were determined as follows: $2^n$ ($2^2 = 4$: cube point) + $2n$ ($2 \times 2$: star point) + 3 (central points: three replicates) [21]. Low and high factor settings (cube points) are coded as −1 and 1, the mid factor settings (central points) coded as 0. The factor settings of trails that ran along axes drawn from the middle of the cube through the center points of each face of the tube (star points) are coded as −1.41 or 1.41 [23]. The ranges and levels of the parameters in the present study are shown in Table 1.

By conducting multiple regression analyses of the experimental data, the following response $Y$ was obtained through the second-order polynomial model:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \qquad (1)$$

where $Y$ contains the dependent variables (the root elongation in study using non-As treated soil and $EC_{50}$ of As in the phytotoxicity study, respectively), $\beta_0$ is the intercept, $\beta_i$ the linear coefficient, $\beta_{ij}$ the interaction coefficient, $\beta_{jj}$ the quadratic coefficient, and $x_1$ and $x_2$ (for Fe and SMS contents, respectively) the coded independent variables. The experimental design of the present study developed mathematical models for interpretation of the root elongation and toxicity of As to lettuce in artificial test soils, consisting of varying combinations of selected soil parameters. The generated model might also be used in simulations to provide useful information on the effects of the Fe and SMS contents on the As toxicity towards plant growth.

### 2.2. Soil preparation and As treatment

Standard artificial soils were used as the test soils, consisting of kaolin clay as the clay type, quartz sand as the sand type, zero-valent iron (ZVI, obtained from Sigma–Aldrich) as the Fe type, and finely ground SMS (obtained from a local mushroom farm) as the OM type [20]. Nine different soil compositions were provided by adjusting the Fe and SMS contents to levels determined by the CCD (Table 1). Soils containing the assigned amounts of Fe (ranging from 0.59 to 3.41%) and SMS (0.59 to 3.41%) were prepared using 20% kaolin clay, with the percentage of quartz sand (74 to 78%) adjusted according to amounts equal to those of the ZVI and SMS added.

100 mM of As solutions, prepared using an appropriate amount of $Na_2HAsO_4$ (Sigma–Aldrich, USA), were used as the stock solution. Appropriate amounts of the stock solutions were added to the test soils to maintain the soil moisture content at 60% of the water-holding-capacity (WHC). The As solutions and test soils were thoroughly mixed to obtain homogeneity, at nominal concentrations of 0, 7, 37, 75, 375 and 749 mg As kg$^{-1}$ dry soil, with three replicates per concentration. The spiked soils were then allowed to equilibrate at room temperature and 60% of the WHC for 8 weeks prior to the root elongation tests.

### 2.3. Phytotoxicity assay with lettuce

The root growth tests with lettuce (*Lactuca sativa* L.) were carried out according to the OECD Guidelines for the Testing of

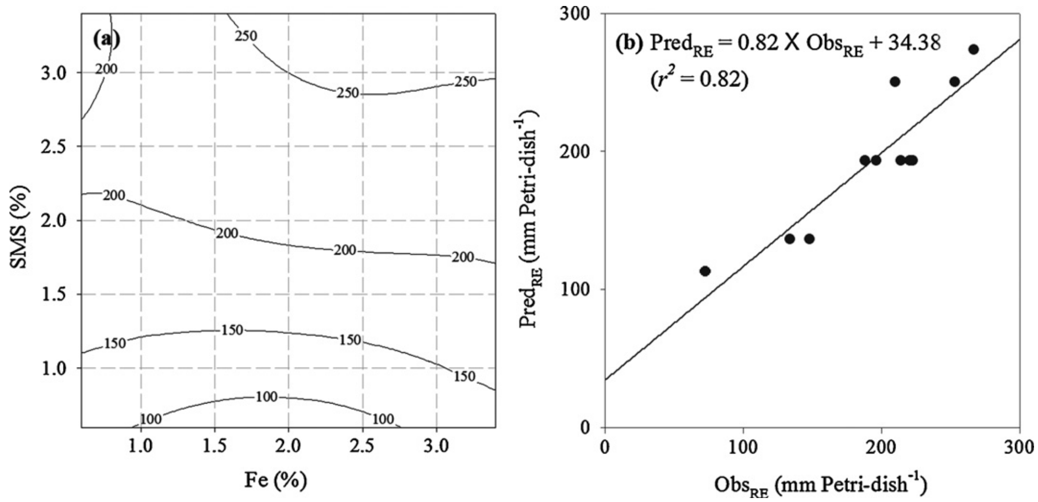

**Fig. 1.** The response surface for the root elongation of lettuce (*Lactuca sativa* L.) in the control soils as functions of the iron (Fe in % dry wt.) and spent mushroom substrate contents (SMS in % dry wt.) (a); and the relationship between the root elongation predicted by the second-order polynomial model (Pred$_{RE}$) and observed root elongation (Obs$_{RE}$) with each combined soil treatment after 21-d (b).

Chemicals (test No. 208: the Terrestrial Plant Test) [24], with small modifications. Prior to sowing, the seeds were sterilized with 10% $H_2O_2$ for 10 min to stimulate germination and prevent fungal growth. Subsequently, ten seeds were placed in 100 × 20 mm plastic Petri-dishes, containing 30 g of the test soils. The Petri-dishes were randomly placed in a growth chamber under controlled conditions. The moisture content was maintained at approximately 60% of the WHC of the soils, with the light conditions of 16 h of daylight and 8 h of darkness per day, at $24 \pm 2\,^{\circ}$C. Three weeks after sowing, the plants were harvested to determine their root growth using an image analyzer program (WinRhizo 5.0a, Regent, Canada). The root elongation was calculated for each Petri-dish based on the sum of root length of plants and expressed as mm Petri-dish$^{-1}$.

After determination of the root growth, the plants were separated into shoots and roots. The roots were soaked in ice cold 0.01 M $NH_4H_2PO_4$ for 15 min to dissociate apoplastic As, rinsed with deionized water [25] and then dried in a fan-forced oven at 60 °C for 48 h. The dried samples of about 50 mg were digested with 3 ml of concentrated $HNO_3$ (60%) for 1 h, using a microwave digester (Milestone, ETHOS Labstation, Italy), and immediately filtered. The filtrates were used to measure the As concentrations of the roots, using an inductively coupled plasma-mass spectrometer (ICP-MS) (Varian, Varian 820-MS, USA). The accuracy of As determination was verified using a certified reference material BCR-402 (white clover materials), which contained 0.093 $\mu$g g$^{-1}$ DW of As. The recovery and coefficient of variance for As determination from quadruplicate samples were 91.1% and 0.03%, respectively.

### 2.4. Statistical analysis

The statistical analyses of the data obtained were performed using SAS v 8.02 (SAS Institute Inc., USA). The $EC_{50}$ values of the root growth on exposure to As were estimated by fitting the data to a 3-parameter logistic regression model [26]. The relationships between the As concentrations and the growth of the roots in a phytotoxicity assay; and between those predicted by the refined models and the observed data (i.e., root elongation in control soil and $EC_{50}$ in phytotoxicity assay) were evaluated using a Pearson's correlation analysis at $p < 0.05$. Two-way analysis of variance (ANOVA) was also performed to test the effects of Fe and SMS on $EC_{50}$ of As for the root elongation of lettuce. To test the adequacy of the second-order polynomial models, a *lack-of-fit* test was used. The parameters and their significances in the second-order polynomial models were tested using a general linear model (GLM).

## 3. Results and discussion

### 3.1. The effect of Fe and SMS on the root growth of lettuce

Table 1 shows the results of the experimental design for the root elongation and percentage of germination used to investigate the effects of the Fe and SMS contents on the growth of lettuce. The average root elongation and percentage of germination in the non-As treated soils ranged from 72 to 266 mm Petri-dish$^{-1}$ and from 93 to 100%, respectively. The germination of lettuce in the non-As treated soils showed no significant difference ($p > 0.05$), whereas the root elongation did ($p < 0.05$).

A model fitting of the results from Table 1 was performed for the experimental design, with a regression analysis with a *lack-of-fit* test used to evaluate the adequacy of the fitted model (Fig. 1)a. The adequacy of the developed model was verified by the high coefficient of determination ($r^2 = 0.94$) with an insignificant *lack-of-fit* test ($F = 1.06$ and $p = 0.52$). The SMS content was the only significant variable ($p = 0.026$) contributing to the response variation of the root elongation of the lettuce in the control soils. This linear factor alone was able to explain 82% of the overall variance of the root elongation in non-As treated soils. Nevertheless, the other linear factor (Fe), as well as quadratic ($Fe^2$ and $SMS^2$) and interaction factor (Fe*SMS), exhibited no significant. Thus, SMS alone remained after the refinement of model as follows:

$$\text{The root elongation of lettuce in non-As treated soil} \times (\text{mm Petri-dish}^{-1}) = 79.39 + 56.99 \times \text{SMS} \qquad (2)$$

This refined model might provide information for judging the important soil parameters influencing root growth, as well as in the prediction of background levels of root growth in control soils consisting of varying combinations of Fe and SMS. This result demonstrated that the root growth of lettuce in non-contaminated soils could be mostly explained as a function of the SMS content. There was also a significant linear relationship ($r^2 = 0.82$) between the model-predicted (Pred$_{RE}$) and observed root elongation (Obs$_{RE}$) (Fig. 1b).

The findings from the response surface model were, to some extent, expected. The increased root growth in the SMS-rich soils could have been the result of enhanced soil fertility conditions,

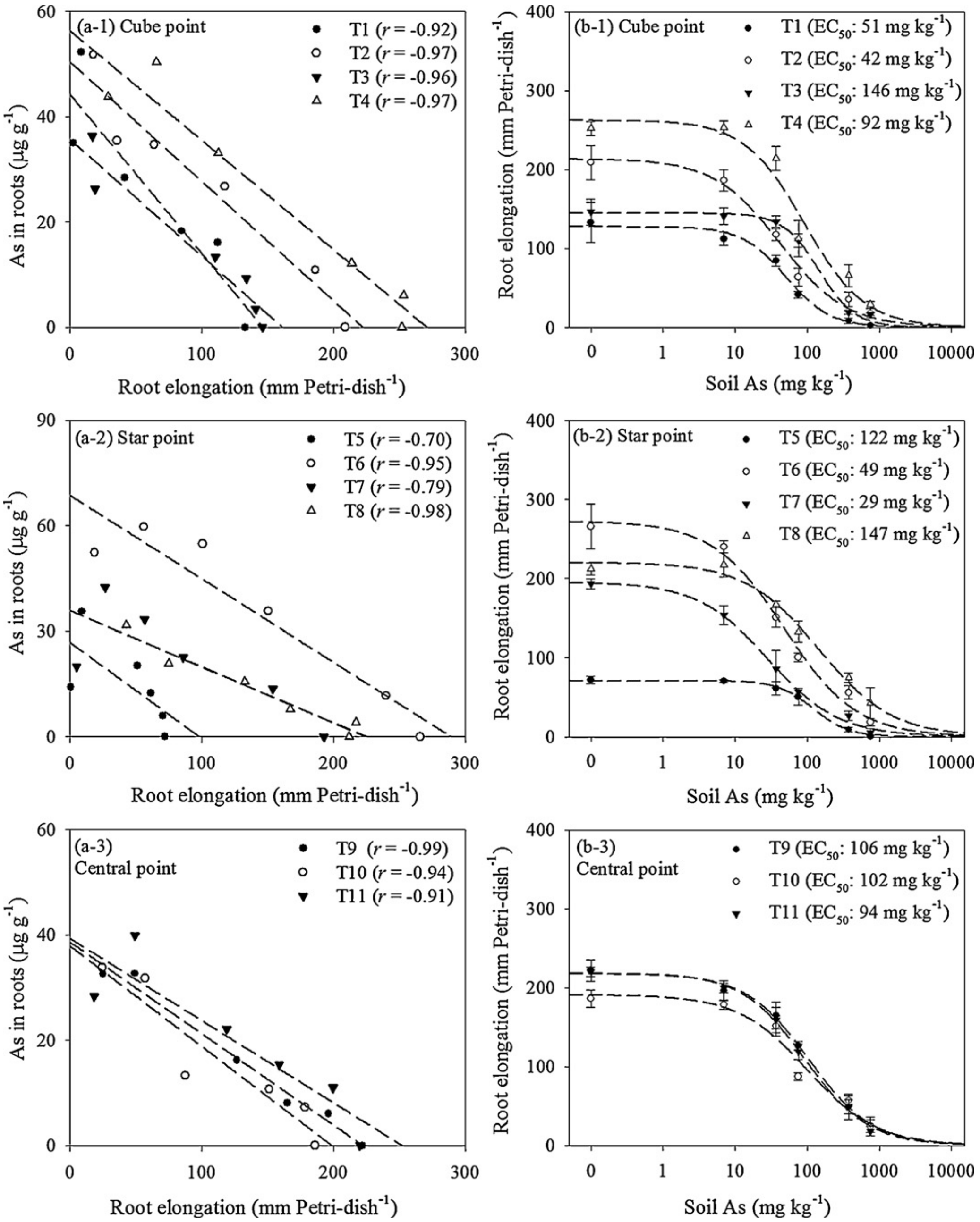

**Fig. 2.** The relationship between As concentrations and the elongation of lettuce (*Lactuca sativa* L.) roots (a); and changes in the elongation of lettuce roots as a function of the As concentration in the test soils (b). [The Cube, Star and Central points signify the experimental points in Table 1; Treatment 1 (T1), Fe application rate (Fe) 1%/spent mushroom substrate application rate (SMS) 1%; T2, Fe 1%/SMS 3%; T3, Fe 3%/SMS 1%; T4, Fe 3%/SMS 3%; T5, Fe 2%/SMS 0.59%; T6, Fe 2%/SMS 3.41%; T7, Fe 0.59%/SMS 2%; T8, Fe 3.41%/SMS 2%; T9, T10 and T11, Fe 2%/SMS 2%; $EC_{50}$, the median effective concentration]

since the applications of organic amendments can improve the soil aeration, structure, nutrient contents and water holding capacities [13,27–29]. In addition, humic acid derived from OM obviously promoted the earliest stages of emergence, elongation and plasma membrane $H^+$-ATPase activity of the plant roots [30].

Unlike the SMS in the refined model, the Fe content was not shown to significantly affect root growth in the control soils ($p = 0.84$), which might have been due to appropriate Fe application rates (ranging from 0.59% to 3.41%) in the present study being beyond the level that limited growth. Generally, Fe is an essential mineral element for the growth of plants [31]. Under low availability of Fe in soils, plants may suffer from Fe deficiency, with their developments then largely inhibited [32]. By contrast, higher Fe application rates (>5%) also may cause problems associating with increasing soil aggregation and compaction, and consequently have no beneficial affect on the induction of plant growth [33]. However, in a hydroponic study on the exposure to elevated concentrations of toxic metals, including Fe, the root elongation of rye grass was the least affected by Fe compared to other elements [34].

### *3.2. Changes in As toxicity on the root growth of lettuce as functions of Fe and SMS contents*

Fig. 2 shows the results of the relationship between As uptake and the elongation of lettuce roots (a); and changes in root elongation as a function of treated As levels in the test soils after 21-d exposure (b). The results indicated that there were significant negative correlations between the As concentration and

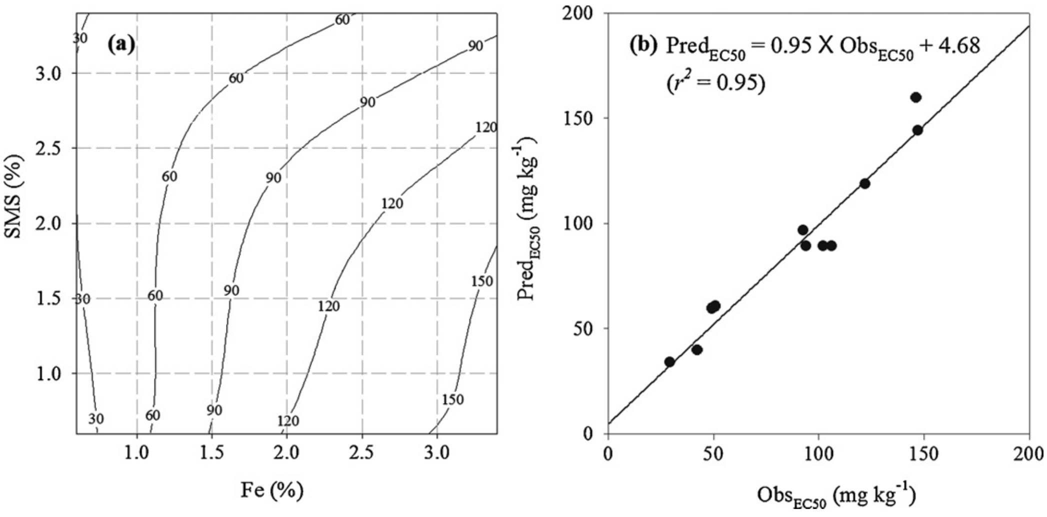

**Fig. 3.** The response surface for the phytotoxicity ($EC_{50}$, the median effective concentration) of As (0–749 mg $kg^{-1}$) towards lettuce (*Lactuca sativa* L.) as functions of the iron (Fe in % dry wt.) and spent mushroom substrate contents (SMS in % dry wt.) (a); and the relationship between the $EC_{50}$ values predicted by the second-order polynomial model ($Pred_{EC50}$) and those observed ($Obs_{EC50}$) for As over 21-d of exposure with each combined soil treatment (b).

lettuce root growth ($r < -0.70$) with all the experimental treatments (Fig. 2a). Although some As-hyperaccumulating plants, such as Chinese brake fern (*Pteris vittata* L.), can uptake inordinate amounts of As without damages to their growth [15], the toxic effects of As on growth of As-sensitive plants are mainly related to As uptake trends [35]. The toxic effects of absorbed As on the growth of plants are due to the oxidative stress induced by the elevated production of reactive oxygen species (ROS), including $O_2^-$ (superoxide radical), $OH^\bullet$ (hydroxyl radical) and $H_2O_2$ (hydrogen peroxide) [1]. However, plants have also detoxification mechanisms to absorbed As. Representative defense mechanisms are enzymatic (including the ROS-scavenger enzymes such as superoxide dismutase, catalase and peroxidase) [36] and non-enzymatic mechanisms (including the synthesis of metal-binding peptides like phytochelatins) [37]. Nevertheless, when these mechanisms are saturated, ROS cause inhibitory effects on plant growth via disruption of the cell membrane and the hindrance of metabolism within the plant cells [1]. However in the present study, no significant differences in the growth of shoots were observed with any of the treatments (data not shown). Similar to our result, some previous reports have found that the uptake and growth of roots were more sensitive to As exposure than those of shoots [35,38]. Therefore, the present study dealt with only the root growth of lettuce.

To take the declared objectives of our experiment and render them into a quantitative measurement [22], the $EC_{50}$ values were estimated and applied in comparison studies on the As toxicity in the test soils. The estimated $EC_{50}$ (ranging from 29 to 147 mg As $kg^{-1}$ dry soil) varied depending on the treated levels of Fe and SMS (Fig. 2b). Two-way ANOVA results also indicated significant Fe ($F = 35.00$ and $p < 0.0001$), SMS ($F = 10.60$ and $p = 0.0001$) and their interactive ($F = 4.89$ and $p = 0.037$) effects for $EC_{50}$ of As on the root elongation of lettuce. The lowest $EC_{50}$ was observed at T7 (Fe 0.59% and SMS 2% contents, respectively), while the highest at T8 (Fe 3.41% and SMS 2%). In detail, under a 2% Fe incorporation rate, the $EC_{50}$ of As in T5 (SMS 0.59%) was 21% higher than the average value (101 mg $kg^{-1}$) of the central points (T9, T10 and T11); whereas T6 (SMS 3.41%) showed a 51% decrease in the value (Fig. 2b). Similar trends were also observed with the 1% (T1 and T2) and 3% (T3 and T4) Fe treatments. However, contrary results were shown under the same SMS conditions. With the 2% SMS treatments, the $EC_{50}$ increased with increasing Fe contents as follows: T7 (Fe 0.59%) < central points (Fe 2%) < T8 (Fe 3.41%). Similar relationships were observed with the other treatments (1 and 3% SMS treatments). Nonetheless, the root elongations, contrary to the $EC_{50}$ values, increased with increasing SMS contents (Fig. 2b). The lowest root elongation of each As-treated level (7, 37, 75,375, and 749 mg As $kg^{-1}$) was observed at 0.59% SMS treatment, while the highest at 3 or 3.41% SMS treatments. The findings from the $EC_{50}$ and root elongation studies demonstrated convincingly that, as mentioned above, SMS in soils could play important roles in plant growth via the enhancement of soil fertility conditions, but could also lead to more rapid increases in inhibitory effects of As on their growth with increasing As concentrations in soils. Briefly, $EC_{50}$ of As increased with increasing Fe contents; whereas, increasing the SMS content, decreased $EC_{50}$ of As despite induction of the root elongation. These results obviously indicate that As influences the root growth of lettuce, and that toxicity levels varied with combinations of the Fe and SMS contents in the test soils.

To interpret and predict the effects of the Fe and SMS contents on phytotoxicity of As, using the response surface methodology, a model fitting of the results from Fig. 2b was performed for the experimental design and the regression analysis ($r^2 = 0.98$) with a *lack-of-fit* test ($F = 2.66$ and $p = 0.29$) used to evaluate the adequacy of the fitted model (Fig. 3)a. From the developed model, the Fe content ($p = 0.0032$) and interaction factor between both parameters ($p = 0.048$) were found to be the significant variables contributing to the response variation in the $EC_{50}$ values of As on root elongation. This linear factor (Fe) alone was able to explain 71% of the overall variance in the $EC_{50}$ value, and the interaction factor (Fe*SMS) explained 23% in the As-treated soils. The other linear factor (SMS) and quadratic factors ($Fe^2$ and $SMS^2$) showed no significance. The following surface response model was derived:

$$\begin{aligned} &EC_{50}\quad \text{of As on the root elongation of lettuce}\quad (\text{mg kg}^{-1}) \\ &= 11.01 + 60.03 \times \text{Fe} - 10.50 \times \text{Fe} * \text{SMS} \end{aligned} \tag{3}$$

This refined model indicated that As toxicity on root growth of lettuce could be predicted as functions of Fe and SMS contents in As-treated soils. Similar to the results derived from Fig. 2b, these results reconfirmed that the soil properties, particularly Fe, relating to the mobility of toxic metal were the important factors affecting their toxicity [8,39]. In fact, Fe oxide has a high affinity for the adsorption of As; therefore, the mobility of As in soil is markedly associated with the presence of Fe oxide on the soil surface [3]. Of the many Fe sources, ZVI is well known and commonly used as a stabilizing agent for As [6,33]. When incorporated into soils, Fe sources are quickly oxidized through several oxidation steps, forming poorly crystalline Fe oxides, with the subsequent adsorption of As via ligand exchange of the As species for the $OH_2$ and $OH^-$ groups on the Fe oxides [7]. For this reason, the mobility of As and its absorption by plant roots in Fe-amended soils decreased and; subsequently, the inhibitory effect of As on root growth might also be reduced.

However, the other significant factor (Fe*SMS), which showed a negative estimated coefficient in the refined model, decreased $EC_{50}$ values on the root elongation of lettuce. It was likely that the As mobility was enhanced by Fe and dissolved organic carbon (DOC) derived from SMS [40]. The DOC in the presence of Fe promoted the formation of soluble As–Fe–organic complexes and prevented As from being adsorbed and precipitated onto Fe oxides as a result of the formation of Fe oxides having been inhibited [6,14]. Especially, Wang and Mulligan [14] reported that the As mobility in OM-treated soils was largely correlated with the Fe concentrations in the column effluents ($r^2 = 0.93$) from a column study using mine tailings and natural organic matter. This report explained that Fe could help to enhance the As mobility by acting as a bridging agent for its incorporation into soluble organic complexes. An alternative explanation regarding the interaction between Fe and SMS concerns the increased pH-triggered dissolution of Fe from Fe oxide surfaces, under the decreased pH conditions of rhizosphere, which would be caused by low molecular organic acids from plant roots [41,42]. This might result in the co-dissolution of the As sorbed onto Fe oxide surfaces due to complexation reactions between DOC, As and Fe [41,43] and; subsequently, induce the uptake of As by plant roots. This complexation reaction could be also enhanced by DOC [42]. As a result, SMS in soils would certainly enhance plant root elongation in As-treated soils as well as non-As treated soils due to the improved soil fertility, but could reduce $EC_{50}$ of As in soils containing elevated concentrations of As.

In addition, a significant linear relationship ($r^2 = 0.95$) was observed between the refined model-predicted ($Pred_{EC50}$) and observed $EC_{50}$ ($Obs_{RE}$) values (Fig. 3b). This indicated that the developed model could provide useful information for predicting the influences of the Fe and SMS contents on As toxicity towards plant growth within a range of tested factors. As expected, the response surface methodology; particularly CCD, was a valuable tool for evaluating the mathematical relationships between selected variables and the responses using minimal experimental sets, without the loss of any statistical reliability [19,22,44].

## 4. Conclusions

The results from the present study confirmed that soil parameters (Fe and SMS contents) were important factors affecting their toxicity. The Fe content in the test soils significantly reduced As toxicity to the root growth of lettuce. However, despite SMS induced the root growth of lettuce in As-treated soils as well as non-As treated soils, SMS in As-treated soils might also cause increases in inhibitory effects of As on the root growth with increasing As concentrations, due to its interaction with Fe. Although the results in the present study are limited to the ranges of soil parameters and artificial test soils studied, the developed response surface models can be used to provide useful information for interpreting phytotoxicity of As in contaminated soils as functions of both parameters. This model together with other related study can also bring about more scientific-based risk assessment for As-contaminated field soils. In addition, the application of this statistical tool can be considered a powerful and efficient technique for the interpretation and prediction of the complicated results caused by the interactions between many factors within the soil environments.

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