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Washing Bottom Sediment for The Removal of Arsenic from Contaminated Italian Coast

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Abstract: Among various forms of anthropogenic pollution, the release of toxic metals in the environment is a global concern due to the high toxicity of these metals towards living organisms. In the last 20 years, sediment washing has gained increasing attention thanks to its capability to remove toxic metals from contaminated matrices. In this paper, we propose a Response Surface Methodology method for the washing of selected marine sediments of the Bagnoli-Coroglio Bay (Campania region, Italy) polluted with arsenic and other contaminants. We focused our attention on different factors affecting the clean-up performance (i.e., liquid/solid ratio, chelating concentration, and reaction time). The highest As removal efficiency (i.e., >30 μg/g) was obtained at a liquid/solid ratio of 10:1 (v/w), a citric acid concentration of 1000 mM, and a washing time of 94.22 h. Based on these optimum results, ecotoxicological tests were performed and evaluated in two marine model species (i.e., *Phaeodactylum tricornutum* and *Aliivibrio fischeri*), which were exposed to the washing solutions. Reduced inhibition of the model species was observed after nutrient addition. Overall, this study provides an effective tool to quickly assess the optimum operating conditions to be set during the washing procedures of a broad range of marine sediments with similar physicochemical properties (i.e., grain size and type of pollution).

Keywords: arsenic removal; sediment washing; citric acid; RSM approach; ecotoxicological assessment

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1. Introduction

Anthropogenic pollution in soils and sediments is considered a global concern due to the capability of most polluting species to accumulate in living organisms [1].

Among the different forms of anthropogenic pollution, the release of toxic metals in environmental matrices is one of the most dangerous due to the high toxicity of these metals towards living organisms, which often exhibit metal bioaccumulating ability [2,3]. Heavy metals can occur in several forms in water and soils, with different degrees of mobility which correspond to different risks [2,4–6]. Arsenic is renowned for the toxicity of its diverse chemical species and widespread contamination via anthropic sources.

Large amounts of arsenic were found in Huangshui Creek (China) up to a maximum value of 120 μg/g [6]. A median concentration of arsenic of 50–190 μg/g was found in rivers of the Hunan province (China) [6]. An arsenic concentration of 6365 μg/g was detected in Portugal due to past and present mining activities and industries [7]. In Italy as well, due to the high levels of toxic metals, some contaminated areas (i.e., Piombino, Priolo, Bagnoli, Falconara Marittima, etc.) were identified as Sites of National Interest (SINs) [7].

At present, among the ex situ technologies aimed to eliminate heavy metals from soils/sediments [8], extraction and/or washing clean-up procedures have received much attention in the last 20 years [9,10]. These physicochemical clean-up techniques have been demonstrated to successfully remove toxic metals (i.e., As) from contaminated matrices at laboratory, pilot, and field trial scales (Table 1). Preliminary separation of the different grain fractions to increase the removal efficiency and reduce the volumes to be treated is required [10]. Among the extracting agents used to transfer metal contaminants from the sediments to the extracting solution, chelating agents are preferred due to their ability to complex metal ions, thus preserving the natural physicochemical properties of the sediment [11]. Ethylene diamine tetra-acetate (EDTA) and ethylene diamine disuccinate (EDDS) have been extensively tested in previous studies as metal complexants [12–16], despite their high costs and low biodegradability. Citric acid (CA) can be considered an interesting alternative chelating agent, due to its biodegradability and enhanced removal capability of heavy metals [17]. Furthermore, exhausted solutions with organic acids, such as CA, can be effectively treated through environmentally friendly processes [18,19]. Lumia and co-workers [9] recently reported a removal efficiency of 30% during sediment washing tests aimed at mercury removal from marine sediments of the Augusta Bay (Sicily region, Italy) in the presence of a high concentration of citric acid (i.e., 1 M). A similar result was obtained for arsenic removal by Nguyen Van et al. [20], who employed citric acid as a chelating agent at a concentration of 200 mM. The physicochemical properties of the target contaminants and the sediments/soils to be treated, the chelating agent, and the experimental conditions adopted (e.g., reaction time, liquid/solid ratio) are the main factors affecting the washing procedure. After assessing a thorough physicochemical characterization of the contaminated matrix and selecting a proper chelating agent, the optimization of the physicochemical process mainly involves the study of the effect of different operating conditions on metal extraction efficiency. Despite the great number of studies dealing with sediment washing, several authors reported only the OVAT (One Factor at A Time) approach to optimize the process [21,22]. However, the OVAT approach has shown several critical aspects, such as the inability to consider the interaction between the different factors affecting the chemical process. Contrary to OVAT, the DoE (Design of Experiment) strategy allows the selection of a minimum number of experiments from which it is possible to (i) understand the effect of the single factors affecting the system and their interaction and (ii) identify the optimum operating conditions for the studied system. DoE is a matrix-based multifactor technique that measures interaction effects and covers the whole multidimensional experimental region [23]. In particular, the use of the Response Surface Methodology (RSM) has been proposed by several authors to understand multivariate phenomena and assess a process scale-up [24–30]. It has significant applications in the design and optimization of processes, as well as in the enhancement of existing designs. For instance, the RSM was recently used for the removal of chromium and cadmium by employing EDTA and poly-aspartate as chelating agents during soil washing, respectively [31,32]. The RSM contains functions and data types for factor-level encoding and decoding. In response surface analysis, appropriate coding is a crucial component. Central Composite Designs (CCD) can be used for plan experiments to identify the variables that have the highest impact on heavy metal removal efficiency.

Herein, for the first time, we propose an RSM method for the washing of selected Italian sediments dragged from the Bagnoli-Coroglio Bay (Campania region). The selected marine sediments were polluted with arsenic and other contaminants. Several operating factors affecting metal extraction efficiency, i.e., liquid/solid ratio (L/S), chelating concentration, and reaction time, were considered. Using response surface methodology (RSM) with central composite design (CCD), we optimized and validated the sediment washing conditions, which is important to scale up lab-based sediment washing studies, reduce cost, and improve the effectiveness of contaminant removal. Based on the results, ecotoxicological tests were performed in two marine model species (i.e., *Phaeodactylum tricornutum* and *Aliivibrio fischeri*), which were exposed to the washing solutions.

Table 1. Summary of the sediment and soil washing treatments for As removal. L/S = liquid-to-solid; As_0 = arsenic at time 0.

Type of Washing Solution	L/S ratio [*w*/*w* or *v*/*v*]	Washing Time [h]	Total As_0 Concentration [µg/g]	As Removal [%]	Ref.
Citric acid at 1.0 M mixed with Rhamnolipid (1.0%) at 2:1	15/1	6.55	<140	84	[33]
Sodium hydroxide at 0.1 M	10/1	24	21,030	96	[34]
Citric acid at 0.2 M and potassium phosphate at 0.1 M	1–5/1	1–2	<12	>95	[35]
Phosphoric (0.05 M)-oxalic (0.075 M) acid-Na_2EDTA (0.075 M) sequence	15/1	0.50	153	42	[36]
Phosphoric acid at 0.5 M	5/1	1	>59	32–62	[37]
2.0 M phosphoric acid, 2.0 M sodium hydroxide or 0.1 M dithionite in 0.1 M EDTA	5/1	24	167	90	[38]
0.5 M oxalic acid	20/1	3	19,100–75,350	70	[39]
0.1–2.0 M of hydrochloric acid or sodium hydroxide	5/1	6	1410	>99	[40]
Sulfuric acid at 0.6 M with 0.6 M phosphoric acid (1:1)	7/1	120	140	71	[41]
Citric acid at 1.0 M	10/1	94.22	52.3	73	Our study

2. Materials and Methods

2.1. Materials

Hydroxylammonium chloride (reagent grade >98% *w*/*w*), ammonium acetate (>99% *w*/*w*), hydrogen peroxide solution (30% *v*/*v*), acetic acid (ACS reagent >97% *v*/*v*), and nitric acid (ACS reagent >67% *v*/*v*) were supplied by Sigma–Aldrich (Steinheim, Germany). Citric acid (99.6%, ACS reagent, anhydrous) was purchased from Thermo Fisher Scientific (New Jersey, USA). Bi-distilled water was used for analytical preparations and dilutions. The sediment samples were collected from the Bagnoli coast (Naples, Italy), then placed in a hermetic plastic box, transported at ambient temperature to the laboratory, dried in a laboratory oven at 40 °C, and finally stored at room temperature. Only samples with a grain size below 2.0 mm was employed for the experimentation (See Figure S1).

2.2. Experimental Setup

The sediment washing experiments were performed in 50 mL polyethylene bottles in batch mode. An extracting solution containing a fixed citric acid concentration (i.e., 5 ÷ 1000 mM) was subsequently added to the bottle and mixed with a certain amount (5 ÷ 25 g) of sediment by adopting an L/S of 5:1 ÷ 10:1 (*v*/*w*). The bottles were stirred using a Kombischüttler KL2 mechanical shaker (Edmund Bühler, Bodelshausen, Germany) at 140 rpm for different contact times (i.e., 8 ÷ 96 h) at room temperature. At the end of each batch test, the samples were carefully withdrawn and centrifuged using an Eppendorf 5804 R, and the solution was filtered through 0.45 µm regenerated cellulose filters before the analysis. All experiments were conducted in triplicate.

2.3. Analytical Methods

The grain size analysis was performed according to the ASTM method (standard test method for particle size analysis of soils) D 422-63 [42]. The metal distribution in the sample was evaluated by a multi-step sequential extraction procedure, as reported by Pueyo et al. [43] and Grotti et al. [44]. This sequential extraction procedure allowed us to evaluate the release of metals from 4 different fractions: (I) exchangeable and weak acid-soluble, (II) reducible, (III) oxidizable, and (IV) residual. Prior to determining the total metal concentration, the samples were oxidized with 1 mL of hydrogen peroxide and 8 mL of aqua regia by mixing HNO_3 and HCl in a 1 to 3 ratio and then mineralized using a microwave oven (MILESTONE, One Touch). During digestion, the temperature increased from room temperature to about 175 °C (±5 °C) and remained at this value for 10 min. The metal concentrations were analyzed through inductively coupled plasma-mass spectrometry (ICP-MS), using NexION 350 ICP-MS Spectrometers by Perkin Elmer (Waltham, MA, USA). As was measured with the AFS-8220 atomic fluorescence spectrometer (AFS) by Jitian (Beijing, China).

2.4. Ecotoxicological Tests

Ecotoxicological tests were carried out according to the ISO guidelines. The *P. tricornutum* inhibition test was carried out according to ISO 10253:2016 by considering a growth rate obtained by normalizing the cell density to that of control groups [45]. The *A. fischeri* luminescence inhibition test was performed according to ISO11348-3:2007, and the luminescence was measured after 30 min of exposure [46]. The toxicity tests were carried out without and with nutrient enrichment after 8 and 96 h of treatment. The toxicity is expressed as the luminescence inhibition percentage in the bacteria *A. fischeri* and as the algal growth inhibition of the diatom *P. tricornutum*.

2.5. Statistical Analysis

As for the RSM approach, Minitab software (Version 19) was used to analyze the experimental data. The data are expressed as effect (%) +/− standard deviation. The analyses of the ecotoxicological tests were carried out using XLSTAT (Addinsoft, Paris, France) and GraphPad Prism (GraphPad, San Diego, CA, USA). The results were considered statistically different whit a *p*-value below 0.05.

3. Results

3.1. Sediment Characterization and Sediment Washing

The results of the granulometric characterization showed that the sediment was mainly sandy (i.e., 89%) and to a lesser extent silty–clayey, thus suggesting that sediment washing could be effectively applied [47].

The metal concentrations are shown in Table 2, and the data were compared with international Sediments' Quality Standards (SQSs), which can be used for both sea and freshwater [48,49]. The Probable Effect Concentration (PEC) and the Threshold Effect Concentrations (TEC) were also considered; PEC and TEC respectively represent the concentration of a specific contaminant below which negative impacts on benthic organisms are rarely expected and the concentration of each substance above which adverse biological effects are frequently found. In this regard, TEC was considered the threshold value. Only As exceeded all the threshold values (i.e., TEC and PEC, Table 2) according to previous studies performed on the same site [50–52]. Thus, As bioavailability was also evaluated. Nevertheless, the high concentrations of Pb and Zn were also considered in the sediment washing process, being above the TEC threshold limit values (Table 2).

Table 2. Metal concentrations in the sediment sample compared to SQSs values.

	As	**Cd**	**Hg**	**Cu**	**Cr**	**Ni**	**Pb**	**Zn**
	[μg/g]							
TEC	9.79	0.99	0.18	31.6	43.4	22.7	35.8	121
PEC	33	4.98	1.06	149	111	48.6	128	459
This study	52.3 ± 1.7	0.4 ± 0.05	0.08 ± 0.02	15.4 ± 0.4	22.2 ± 0.9	6.1 ± 0.7	85.2 ± 3.8	237.0 ± 5.9

The results of the bioavailability analyses indicated a distribution of 5%, 31%, 5%, and 59% of arsenic in the sequentially extracted I, II, III, and IV fractions (Table 3), respectively. The dominance of As in the residual fraction might be due to the volcanic nature of the site [53–55]. However, the high content of As in the bioavailable fraction (calculated as the sum of the first three fractions) proved that As is potentially dangerous, as it can be released into the environment. Moreover, both Pb and Zn were higher than the PEC value. Table 3 shows that the quantity of metals was more bound in Fraction 2 than in Fraction 3. This phenomenon could be due to the reduced presence of organic species in the marine sediments considered in the present study.

Table 3. Sequential extraction results. The four fractions are exchangeable and weak acid-soluble (I), reducible (II), oxidizable (III), and residual (IV).

Metal	I Fraction	II Fraction	III Fraction	IV Fraction
	[%]			
Pb	10%	39%	19%	33%
Zn	6%	33%	14%	46%
As	5%	31%	5%	59%

As for arsenic (As), almost half of the total metal concentration identified through the sequential extraction was associated with the first three fractions. It is also possible to observe that in the first two fractions, 49% and 39% of Pb and Zn were observed (sum of the Fractions I and II), respectively. These results confirmed the possibility of issuing the contaminants and, therefore, the applicability of soil washing as a reclamation technique. In this study, we chose CA as an extracting agent, in agreement with Shi et al. [56]. Indeed, these authors reported remarkable extraction efficiencies in the exchangeable and reducible fractions by employing CA.

3.2. Response Surface Methodology Approach

The sediment characterization revealed an exceeding upper limit of both PEC and TEC (i.e., 33 and 9.79 μg/g, respectively) (Table 2) only for arsenic. Therefore, As was selected for the RSM analysis. The optimal working conditions were also examined for the concentrations of the other metals. An RSM was performed to (1) evaluate the relationship between the response (metal extraction) and the main factors affecting the process, (2) understand the effect of the single factors separately at varying levels, and (3) optimize the response with a minimum number of experiments. A CCD face-centered approach was used to estimate the first- and second-order coefficients. The following factors were considered to affect metal extraction (i.e., the response): (i) the reaction time, t (h), (ii) the L/S, and (iii) the concentration (mM) of CA, here used as chelating agent. The low (i.e., −1) and high values (i.e., +1) of the factors were chosen based on previous literature information [9,57] (see Table 4 for coded and un-coded values and levels for each factor). Based on the above-mentioned factors and levels, the software was able to suggest a minimum number of experiments with which it was possible to accomplish the above-reported tasks (1), (2), and (3) (Table 5).

Table 4. Coded and un-coded values and levels of the factors.

Factor	Unit	Level		
		−1	**0**	**1**
Reaction time, t (A)	h	8	52	96
L/S ratio (B)	-	5	7.5	10
Citric acid concentration, CA (C)	mM	5	502.5	1000

Table 5. Design table for the chosen factors and statistical factorial design. The coefficient of determination (R-squared) is equal to 98.73%.

Run	A	B	C	Extracted As (μg/g)	Calculated Response (μg/g)	Run	A	B	C	Extract (μg/g)	Calculated Response (μg/g)
1	0	0	−1	0.00	−0.17	31	0	0	0	19.30	18.64
2	0	0	−1	0.00	−0.17	32	0	0	0	17.73	18.64
3	0	0	0	19.44	18.64	33	0	0	1	30.33	29.78
4	0	0	0	20.00	18.64	34	1	1	1	37.60	39.29
5	0	0	0	19.00	18.64	35	1	−1	−1	0.10	0.55
6	−1	0	0	11.00	11.71	36	1	0	0	19.44	19.12
7	−1	−1	−1	0.00	−1.79	37	1	0	0	20.33	19.12
8	−1	1	−1	0.05	1.37	38	−1	−1	1	20.50	20.07
9	0	0	0	18.43	18.64	39	0	0	0	19.00	18.64
10	1	−1	−1	0.13	0.55	40	0	0	1	29.00	29.78
11	−1	1	1	26.66	26.84	41	−1	−1	1	20.00	20.07
12	−1	1	−1	0.05	1.37	42	0	1	0	27.70	24.25
13	−1	−1	1	20.19	20.07	43	1	1	−1	1.45	1.25
14	0	0	0	18.90	18.64	44	0	−1	0	17.50	20.52
15	1	−1	1	36.39	34.98	45	1	1	−1	1.00	1.25
16	0	0	0	18.90	18.64	46	0	0	0	18.96	18.64
17	0	−1	0	17.58	20.52	47	1	0	0	21.00	19.12
18	0	0	0	18.61	18.64	48	−1	−1	−1	0.001	−1.79
19	−1	−1	−1	0.00	−1.79	49	1	−1	1	36.00	34.98
20	−1	1	−1	0.001	1.37	50	0	0	0	18.04	18.64
21	−1	0	0	11.00	11.71	51	0	0	0	17.89	18.64
22	0	0	0	17.69	18.64	52	1	−1	1	36.50	34.98
23	0	1	0	27.00	24.25	53	1	−1	−1	0.09	0.55
24	0	0	0	17.98	18.64	54	−1	0	0	10.00	11.71
25	1	1	−1	0.75	1.25	55	0	1	0	26.80	24.25
26	0	0	1	29.80	29.78	56	1	1	1	37.80	39.29
27	0	−1	0	18.00	20.52	57	0	0	0	18.00	18.64
28	−1	1	1	28.00	26.84	58	0	0	0	18.20	18.64
29	1	1	1	37.00	39.29	59	0	0	−1	0.00	−0.17
30	0	0	0	18.99	18.64	60	−1	1	1	27.12	26.84

The second-order response of the system Y (i.e., extracted arsenic, μg/g) can be expressed by a polynomial function (Equation (1)), as follows:

$$Y = a_0 + a_1 A + a_2 B + a_3 C + a_{11} A^2 + a_{22} B^2 + a_{33} C^2 + a_{12} AB + a_{13} AC + a_{23} BC \quad (1)$$

where:

- a_0 is the average response;
- a_1, a_2, and a_3 are the principal effects of the studied factors (A, B, C);
- a_{11}, a_{22}, and a_{33} are the effects of the second-order terms;
- a_{12}, a_{13}, a_{23}, and a_{123} are the interaction effects between the factors.

In particular, the response Y (i.e., extracted As, μg/g) obtained from the experimental data using uncoded units, is reported below (Equation (2)). The goodness of the equation was confirmed by the coefficient of determination (R-squared), which was 98.73%; this result was similar to those reported in the literature by others for soil washing [27,31]. The experimental (three replicates) and the calculated responses (in terms of metal extracted) are presented in Table 5, in which it is clear that the mathematical regression model was in good agreement with the experimental data.

$$\begin{gathered} Y = 23.07 + 2.27\cdot 10^{-1}t + 8.30\cdot (L/S) + 3.28\cdot 10^{-2}\cdot CA + 1.67\cdot 10^{-3}t^2 + 5.98\cdot 10^{-1}(L/S)^2 + 1.5\cdot 10^{-5}(CA)^2 + 5.60\cdot 10^{-3}t\cdot (L/S) \\ + 1.44\cdot 10^{-4}t\cdot (CA) + 7.24\cdot 10^{-4}(L/S)\cdot (CA) \end{gathered} \quad (2)$$

The normal probability plot for metal extraction (μg/g) is illustrated in Figure 1. From the evidence that all points are distributed close to the straight line in Figure 1, further proof of a reliable agreement between experimental data and mathematical model could be deduced.

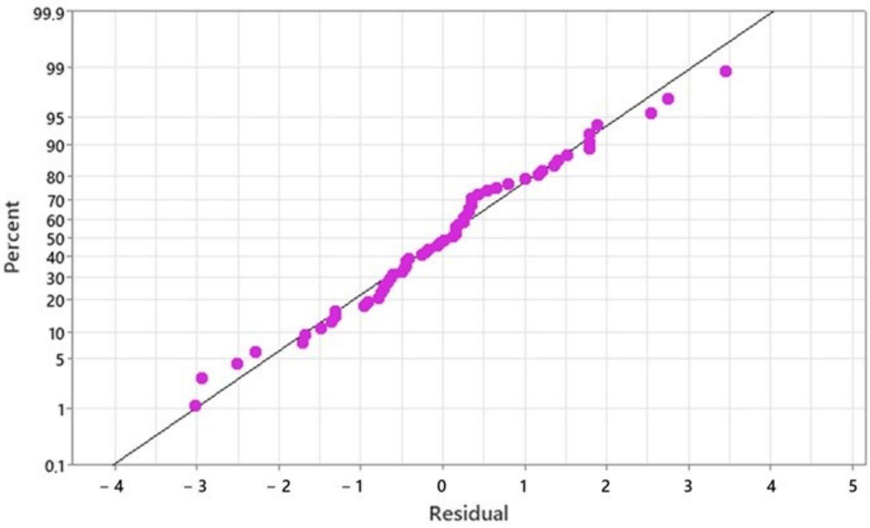

Figure 1. Normal probability plot of the residuals for metal extraction.

Moreover, the effects of the parameters (Factors A, B, C) and their interaction on the response were assessed by using a Pareto chart (Figure 2), which enabled the identification of the relative significance of each factor [58]. Among these values, it emerged that a higher value recorded on the diagram corresponded to an increasing effect of one factor or of the interaction between different factors. The Pareto chart analysis suggested that the citric acid concentration (Factor C) greatly influenced the metal extraction. In contrast, the other factors slightly affected the removal efficiency of arsenic. In particular, among the three factors, factor B affected less the metal extraction compared to A (reaction time) and C (citric acid concentration). This is an interesting result, due to the possibility to reduce the volume of the sediment-washing solution without influencing the metal extraction. Moreover, contour plots were used to fully define the effect of each factor, along with their interactions (Figures 3–5), alternately fixing the medium value of one of the factors.

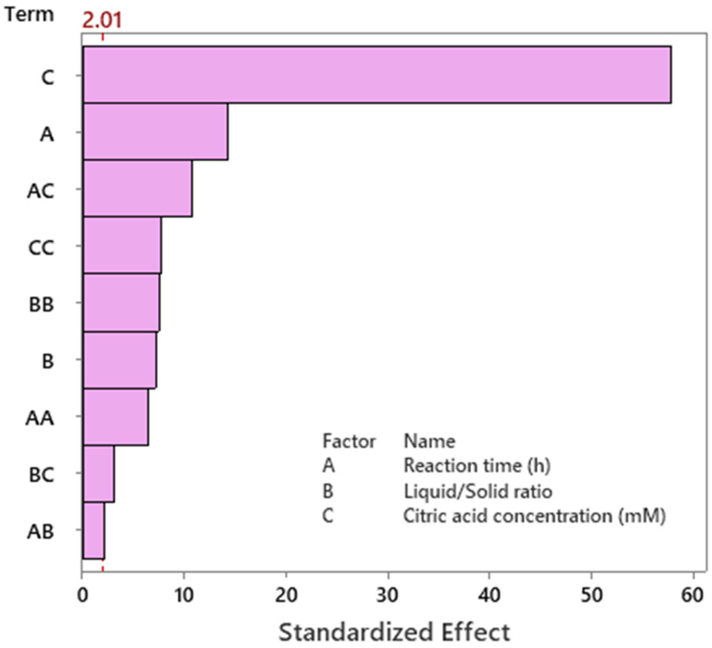

Figure 2. Pareto chart for standardized effects, considered as the response for extracted arsenic (μg/g). Significance level α = 0.05. Factors: A—Reaction time (h); B—Liquid-to-solid ratio; C—Citric acid concentration (mM).

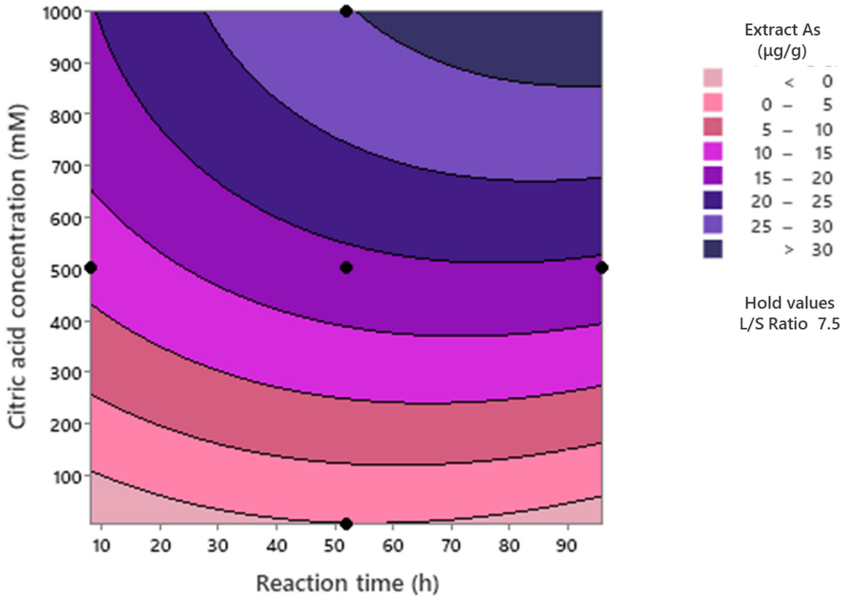

Figure 3. Contour plot of extracted arsenic for the interaction between citric acid concentration and reaction time. The liquid-to-solid ratio was fixed at 7.5.

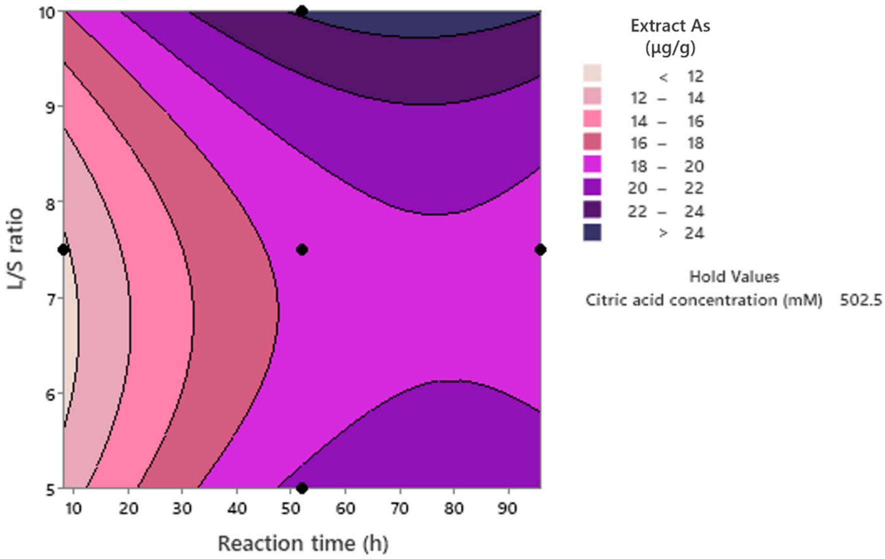

Figure 4. Contour plot of extracted arsenic for the interaction between liquid-to-solid ratio and reaction time. The citric acid concentration was fixed at 502.5 mM.

More in detail, Figure 3 shows the combined effect of citric acid concentration and reaction time when the L/S was fixed at 7.5. As shown in Figure 3, in this case the highest removal efficiency was obtained when the citric acid concentration was higher than 500 mM for a reaction time above 10 h under this operating condition (i.e., L/S = 7.5). Afterwards, the simultaneous effect of the L/S ratio and the reaction time was evaluated by fixing the citric acid concentration at 502.5 mM. As indicated in Figure 4, the highest efficiencies were recorded with an L/S ratio above 9 and a reaction time ranging between 50 and 90 h. This latter effect can be associated with a decreased influence of the reaction time on metal extraction, as reported by the Pareto chart analysis.

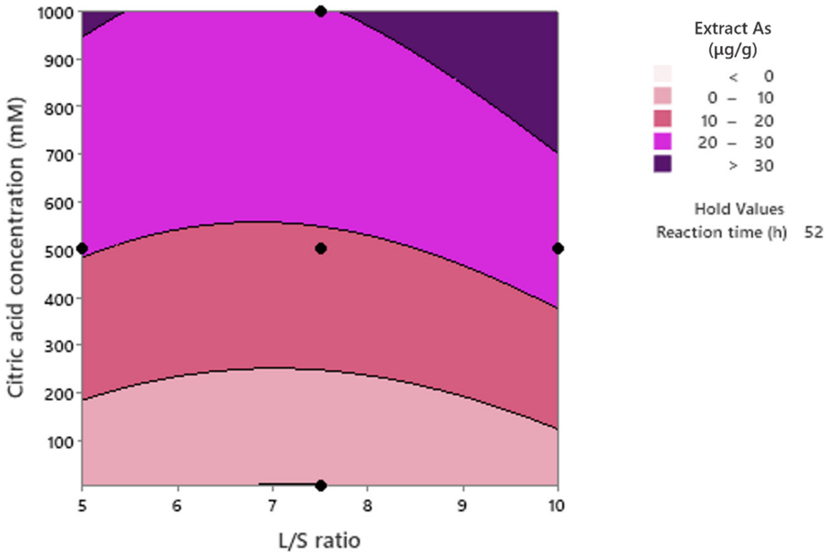

Figure 5. Contour plot of extracted arsenic for the interaction between liquid-to-solid ratio and citric acid concentration. The reaction time was fixed at 52 h.

Moreover, Figure 5 shows the effect of the interaction between citric acid concentration and L/S, when the reaction time was fixed to 52 h. As indicated in the diagram, the maximum removal of arsenic was obtained when the highest values of both L/S and citric acid concentration were used, as previously reported in a literature survey [16]. Therefore, a positive effect for the single factors and their interaction was recorded.

Finally, the optimum values of reaction time, L/S ratio, and citric acid (CA) concentration were estimated by a response optimizer. Three replicate experiments were performed by fixing the optimal values for each factor to verify the predicted conditions. A reaction time of 94.22, an L/S of 10, and a citric acid concentration of 1000 mM resulted to be the best operating conditions. Regarding the reaction time, no improvements in metal extraction were observed beyond the estimated optimum value.

3.3. Comparing the RSM and OVAT Approaches

With the aim to evaluate the effectiveness of the RSM approach and further relate the outcoming trends to the arsenic removal efficiency, the most relevant experimental results are reported and discussed below. The effect of each variable was evaluated by adopting an OVAT approach, and a comparison between experimental and predicted responses was carried out. The main results are reported in Figure 6A–D.

As clearly shown in Figure 6A–C, upon increasing the citric acid concentration, a higher arsenic extraction was observed in all cases. Moreover, a moderate increase in arsenic extraction was obtained upon increasing the L/S ratio (Figure 6A,B), thus confirming the results reported in the previous section (See Figure 2). By comparing the experimental results with those predicted by the statistical analysis, it emerged that the RSM approach was able to describe the system with varying operating factors. As far as the extraction kinetic is concerned, the effect of the reaction time on the extraction of arsenic from sediments is shown in Figure 6D at fixed values of L/S ratio (i.e., 10) and CA concentration (i.e., 1000 mM). Consistent with the general trend reported by others [21,22], the As extraction gradually increased with time until reaching an asymptotic value at 96 h.

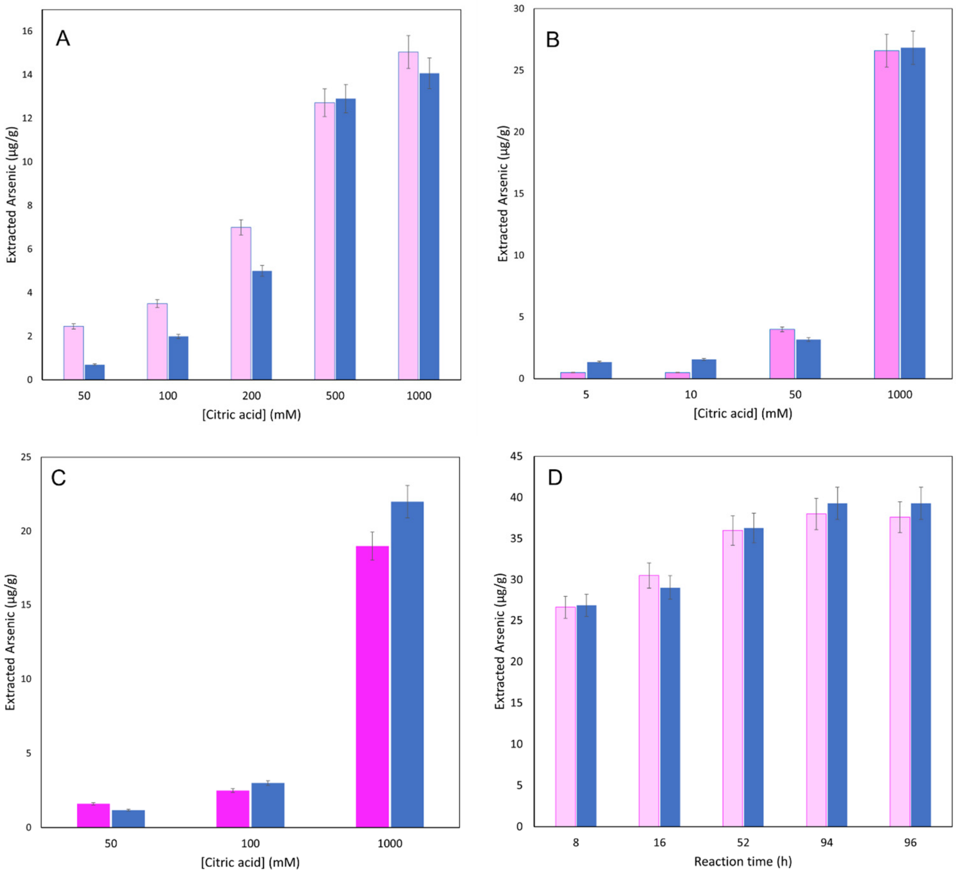

Figure 6. Extracted arsenic at a varying citric acid concentration, (■) experimental and (■) predicted results. (**A**) L/S = 10; reaction time = 8 h; (**B**) L/S = 5; reaction time = 8 h; (**C**) L/S = 5; reaction time = 16 h; (**D**) L/S = 10; citric acid concentration = 1000 mM.

As discussed before, the highest arsenic removal efficiency (i.e., 38 µg/g) was obtained by fixing the highest values of L/S (i.e., 10:1) and citric acid concentration (i.e., 1000 mM). On the other hand, the optimum value for the reaction time was 94.22 h (see Table 1 for a comparison with other previous studies). Likewise, an L/S of 5:1 by fixing the chelating agent at 1000 mM led to an As extraction of more than 30 µg/g after 52 h (Figure 5). The high concentration of citric acid required to improve the removal efficiency can be attributed to (I) the high content of the chelating agent, which increased the arsenic extraction, and (II) the lower pH values obtained, which improved the metal solubility in the washing solution. Therefore, the latter condition can be identified as optimal for practical applications, since a smaller reactor can be used for the sediment-washing procedure. Consequently, a lower amount of spent washing solution (i.e., a half volume) will be treated downstream of the washing process [1]. Therefore, in the two optimum washing conditions (i.e., L/S = 5; [CA] = 502.5 mM; t = 52 h and L/S = 10; [CA] = 502.5 mM; t = 52 h) the concentrations of Pb and Zn were evaluated. At L/S = 5, concentrations of 49.5 µg/g and 172.2 µg/g were calculated for Pb and Zn, respectively. At L/S = 10, concentrations of 35.3 µg/g and 161.4 µg/g were estimated for Pb and Zn, respectively. With respect to the distribution of the sequential extraction reported in Section 3.1, it can be observed that the amount of arsenic removed was of the same orders of magnitude as in the first two fractions. Furthermore, since the arsenic amount did not fall within the PEC values and since the metals bound to the fractions could be defined as "available" [52], ecotoxicity tests were carried out to evaluate the effectiveness of the washings process. Furthermore, it can be observed that the weight loss between the initial sample and the sample treated in the best operating conditions was lower than 3%. This last result proved that the sediment washing treatment is feasible, as the dissolution of toxic metals does not compromise the physicochemical properties of the marine sediments studied.

3.4. Ecotoxicological Tests

Changes in ecotoxicity during sediment washing treatments were evaluated in the washing solutions in the following selected conditions: L/S and chelating concentration of (A) 5:1 and 50 mM, (B) 5:1 and 500 mM, (C) 5:1 and 1000 mM, (D) 10:1 and 50 mM, and (E) 10:1 and 500 mM, respectively. These values were selected by comparing the optimum operating conditions for sediment washing (C) with other conditions for As removal (A, B, D, and E) after 8 and 96 h, without and with nutrient enrichment (Figure 7A,B).

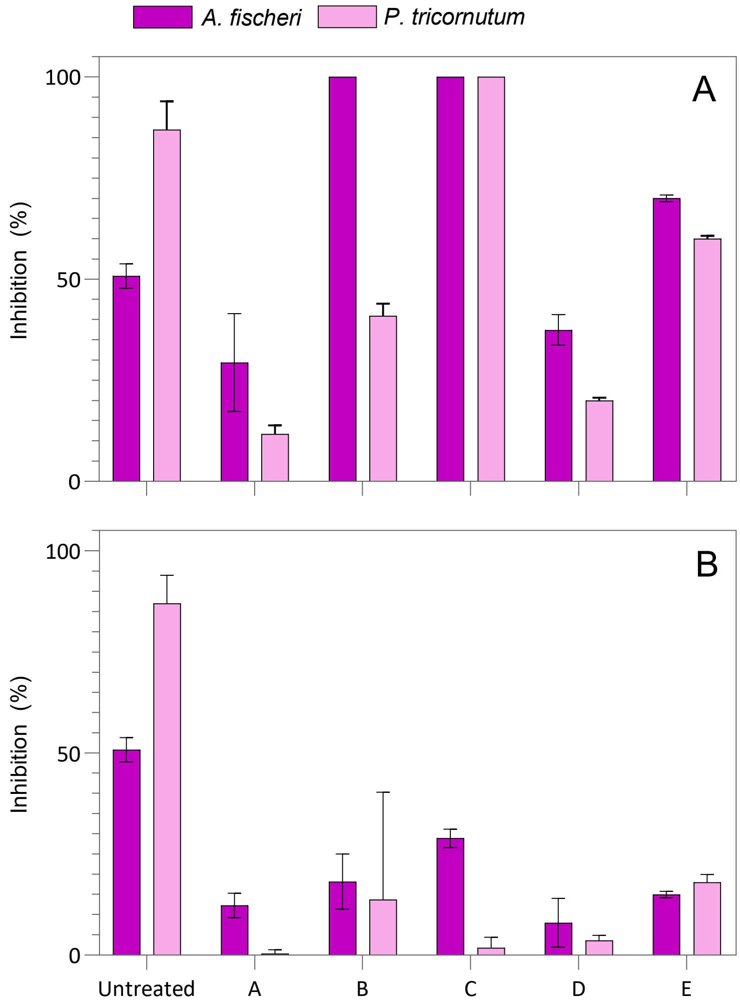

Figure 7. Mean inhibition of *A. fischeri* (purple bars) and *P. tricornutum* (pink bars) after exposure to treated or untreated spent sediment washing solutions. (**A**) Solutions without nutrient enrichment; (**B**) solutions with nutrient enrichment. A = L/S of 5:1 and citric acid at 50 mM; B = L/S of 5:1 and citric acid at 500 mM; C = L/S of 5:1 and citric acid at 1000 mM; D = L/S of 10:1 and citric acid at 50 mM; E = L/S of 10:1 and citric acid at 500 mM.

The results shown in Figure 7A indicate that the initial percentage of luminescence inhibition after 8 h was around 51%, likely due to the presence of As. In addition, the solution toxicity was statistically higher ($p < 0.05$) than the initial toxicity value after 8 h (i.e., 100% of inhibition, Figure 7A), when concentrations of 500 and 1000 mM of citric acid were employed with an L/S of 5:1. On the other hand, the toxicity variation was negligible ($p > 0.05$) if compared with that of the untreated sediment when citric acid (i.e., 50 mM) was used at L/S of 5:1 and 10:1 (Figure 7A). In addition, the data after 96 h of treatment (data not shown) did not reveal significant differences ($p > 0.05$) if compared with those after 8 h of treatment (Figure 7). These results suggest that the chelating agent concentrations mainly affected the toxicity towards living organisms if compared with the effect of the L/S (Figure 7) and the time exposure. On the contrary, Kos and Leštan [59] reported an improvement in the respiration of soil microorganisms with an increase in citrate concentration. This discrepancy can be attributed to the species of the organism used for the ecotoxicological test.

Indeed, *P. tricornutum* is more sensitive than *A. fischeri* in the untreated sediment, showing a 90% of inhibitory effect. This inhibitory effect decreased (Figure 7A) by washing the sediment with 50 and 500 mM citric acid and an L/S of 5:1 (i.e., to 12 and 41%, respectively) or 10:1 (i.e., to 20% and 60%, respectively). In each case, the C condition (i.e., with 1000 mM of citric acid) still showed the highest inhibition (Figure 7A) probably due to the lack of some essential nutrients in the washed solutions after treatment, as reported in other works [57]. The treatment could not have removed the toxicity in the washed sediment for the simultaneous removal of metal and micronutrients combined with an acidic pH, thus indicating its continuous toxicity for bacteria. For this reason, the ecotoxicological tests were repeated with nutrient-enriched sediment and pH adjustment (Figure 7B).

The toxicity significantly decreased in the enriched washing solutions due to a luminescence inhibition ranging from 8 and 29%, while the growth inhibition ranged from 0.4 and 14%, thus indicating that the residual toxicity of the remediation process was mostly due to the lack of leached micronutrients with an acidic pH. Hence, the addition of nutrients in the washing solutions demonstrated that the amount of bioavailable toxic metals in the leachates of the remediated sediments was relatively low despite initial technical issues, as reported above.

4. Conclusions

A sediment-washing process using citric acid as a chelating agent to remediate sandy metal-polluted marine sediments was investigated. RSM resulted to be a suitable method to evaluate the effects of operating parameters (i.e., L/S, chelating agent concentration, washing time) on As removal from contaminated marine sediments. This method can be used to quickly determine, with a small number of experiments, the most influential factors and conditions capable of optimizing the As removal efficiency for sediments with similar grain size composition and contamination levels. By considering the significance deduced for each variable, the set of operating conditions which enabled to achieve the highest As removal efficiency (As removal above 30 µg/g) during the sediment washing was the following: L/S = 10:1 (v/w), citric acid concentration = 1000 mM, washing time = 94.22 h. This optimum condition could be further optimized with a lower L/S equal to 5:1, without influencing the metal extraction, to minimize the volume of spent washing solution to be subsequently treated. In these conditions, the chelating agent consumption and reactor volume could be reduced by approximately 50%. The reduced inhibition of *P. tricornutum* and *A. fischeri* after nutrient addition during the ecotoxicological tests further supports the RSM results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11030902/s1, Figure S1: Overview of operating phases involved before the soil washing experiments.

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