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APPLICATION OF RESPONSE SURFACE METHODOLOGY (RSM) FOR OPTIMIZATION OF ZINC EXTRACTION FROM ANAEROBIC SEWAGE SLUDGE

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Abstract

The reuse of sewage sludge will be increasing in the future, with expansion of sewerage works and advanced sewage treatments. During anaerobic digestion of sewage sludge from wastewater treatment plants the humification of organic matter will have an important effect on the physico-chemical forms of heavy metals. The aim of our paper was to investigate mobility of zinc from dried anaerobic sludge (DANS) from municipal wastewater treatment plant (WWTP) via single step (simultaneous) extraction protocols. We tried to optimize extraction process as a function of amount of sludge biomass, concentration of extracting agent and reaction time. The three parameters namely 0.55 g of anaerobic sludge, 50.5 mmol/dm^3 HCl as extracting agent and 3 hour of reaction time were chosen as center points. The experimental data on extractable Zn concentration (c_{Zn}) from DANS were obtained by stripping chronopotentiometry and fitted into a quadratic polynomial model using multiple regression analysis. The optimal extraction parameters were studied using experimental Box-Behnken design under response surface methodology (RSM). Results showed that interaction effect of reaction time and amount of biomass to Zn extraction is minimal, but in combination with the concentration of HCl significant increase of extractable zinc from DANS was detected. The maximum metal solubilization was obtained at 0.55 g of DANS, 100 mmol/dm^3 HCl and 5 hour of reaction time. Correlation among to predicted and experimental data showed ability to reducing the number of total experiments from 39 to 17.

Key words: DANS, extraction, RSM, stripping chronopotentiometry, zinc

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1. Introduction

The production of sewage sludge from wastewater treatment plants have been increased due to rapid industrialization and urbanization. Unsettleable form and high water content can be one of the problems of sewage sludge disposal. Sewage sludge can be stabilized by anaerobic digestion and dewateration before its deposition in landfills. The feasibility of adequate sites and the risk of contamination of superficial and subterranean waters are some of the drawbacks of sludge disposal on land (Marchioretto et al., 2002). The risk of the sludge utilization is mainly due to pathogens, persistent organic pollutants and heavy metals content. Metal contaminants are particularly problematic because, unlike most organic contaminants, they are non-biodegradable and can be bioaccumulated in living organisms, thus becoming concentrated throughout the food chain (Shrivastava et al., 2003). Metals concentrations in sewage sludge vary from one site to another, depending on municipal and industrial input into system. Binding of metals into organic matrix, organic mineral aggregates and inorganic particles and to carbonates or manganese oxides affect the reversibility of bound and leaching of toxic

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component from sewage sludge into environment. Sludge as an organic matter rich material with low toxic metals concentration can be utilized as fertilizer and soil conditioner in agriculture (Frišták et al., 2013). The benefit of biosolids application to land is to supply and recycle organic matter and nutrients (Martinez et al., 2003). The ecological and toxicological effect of potentially toxic metals from sewage sludge on agro-ecosystem is directly related to their partitioning between mobile and complexed species in the highly heterogeneous soil system (Merrington et al., 1997). Many factors influence this partitioning of toxic metals in soil amended with sludge including the soil and sludge characteristics.

The extraction of toxic metals from the sewage sludge, before land application, is therefore a necessary step for reducing the adverse effects of biosolids application (McBride, 2003). For extraction of heavy metals from sewage sludge a variety of single and sequential extraction techniques can be used. In recent years there has been a great interest for the methods of toxic metals removing from sewage sludge (Babel and Dacera, 2006; Frišták et al., 2012a; 2012b; Marchioretto et al., 2002). These methods can be clasified as a: chemical extraction, using inorganic acids (Marchioretto et al., 2002), organic acids (Veeken and Hamelers, 1999), alkaline reagents (Hsiau and Lo, 1997), chelators (Lo and Chen, 1990), bioloeaching (Pathak et al., 2009), electroreclamation (Wang et al., 2005) and advanced biosolids treatment methods (Dewil et al., 2006). Chemical extractions as a tool for Zn removal from sewage sludge were extensively descripted in work Shiro and Tahei (2000). Zhang and Zhang (2012) showed the application of [S, S]-EDDS as extracting agent for Zn extraction from sewage sludge. Zn extraction by organic acids provides some disadvantages (Babel and Dacera, 2006). Our previous paper (Frišták et al., 2012b) found out, that inorganic extractants such as hydrochloric or nitric acid solutions remove the Zn from sludge structures with highest efficiency. For differences in biosolids structures, extracting parameters and using only limited number of chemical extractants, it is difficult to perform comparison among experimental data and find out the optimal conditions of extraction protocols.

In the paper, we tried to optimize chemical extraction efficiency of zinc from dried anaerobic sludge (DANS) of municipal wastewater treatment plant (WWTP) via single step (simultaneous) extraction protocols using stripping chronopotenciometry. The optimal extraction parameters were studied using experimental Box-Behnken design under response surface methodology (RSM). RSM is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response (Box and Draper, 1987). The methodology is more practical as it arises from experimental methodology which includes interactive effects of the variables and, eventually, it depicts the overall effects of the parameters on the process (Bas and Boyaci, 2007).

The main aim of paper was to describe the effect of amount of sludge biomass, concentration of extracting agent and reaction time on zinc extraction from DANS. Therefore, three level three factor (3^3) quadratic polynomial model under Box-Behnken design (BBD) was developed to predict the relationship between the experimental variables on the total of zinc extracted (response variable) from DANS. Obtained results are useful for application of anaerobic sludge as a soil amendment and conditioner.

2. Material and methods

2.1. Preparation of sludge sample

Municipal anaerobic sludge was obtained from mechanical-biological WWTP in Zeleneč (Slovak Republic). After repeated washing in deionised water, sludge biomass was oven dried at 105°C for 48 h, ground, sieved and stored in closed bottles at 22°C. For extraction experiments fraction < 2 mm was used.

2.2. Extraction experiments

Leachable and bioavailable fractions of zinc from dried anaerobic sludge (DANS) were obtained after one-step extractions with HCl of variable concentrations. HCl as extraction agent with highest extraction efficiency for Zn and anaerobic sludge was used (Frišták et al. 2012b).

Extraction experiments were carried out by suspending of DANS (10 g/dm^3 d.w.) in 10 cm^3 of extraction agent. Flasks were agitated on a reciprocal shaker (150 rpm) at 22°C. After centrifugation (5 min, 5000 rpm) the supernatant was obtained and concentration of zinc was determined by electrochemical analysator. All experiments were carried out in triplicates. Amounts of DANS, concentrations of HCl and reaction time were set according to experimental design methodology.

2.3. Experimental design and optimization of zinc extraction

Variable number of factors such as reaction time, amount of DANS and concentration of HCl as extracting agent can significantly affect the total zinc extraction efficiency. Therefore, Box-Behnken design (BBD) under RSM was used to identify the relationship between the response variable (total extracted zinc concentration) and the experimental variable (amount of sludge biomass, concentration of extracting agent and reaction time).

The statistical software Statgraphics Centurion XV (StatPoint Inc., USA) was used for the experimental design, data analysis, quadratic model building, graph plotting (three-dimensional response surface and contour plots) and to estimate the responses of amount of sludge biomass, concentration of extracting agent and reaction time. The range of experimental variables and their units and notation are indicated in Table 1.

Table 1. Experimental range and levels of independent variables for Zn extraction

Independent variables	*Symbols*	*Unit*	*Coded levels*		
			-1	*0*	*+1*
m_{DANS}	A	mg	100	50	1000
c_{HCl}	B	mmol/dm^3	1	50.5	100
T	C	h	1	3	5

The response variable c_{Zn} (total extractable Zn concentration) can be expressed as a function of the independent experimental variables according to the following RSM guadratic model (Eq. 1):

$$c_{Zn} = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{i \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where: c_{Zn} is predicted response (total extractable Zn concentration); β_i, β_{ii}, β_{ij} - linear, quadratic and interaction effects; β_0 - the constant coefficient; x_i, x_j, …x_k - the input variables (m_{DANS}; c_{HCl}; t) which affect the c_{Zn} value; ε - the error.

In the optimization procedure we studied the response of the statistically designed combinations, estimated the coefficients by fitting the experimental data to the response functions and predicted the response of the fitted model. The adequacy of the model and statistical significance of the regression coefficients were checked using the analysis of variance (ANOVA).

2.4. Galvanostatic stripping chronopotentiometry and pretreatment of samples

Extractable zinc was measured by galvanostatic stripping chronopotentiometry on macroporous electrode E104 L using electrochemical analyzer EcaFlow model GLP 150 (Istran, Ltd., Bratislava, Slovak Republic). Aliquots (5 ml of extracts) were added to flasks with 20 ml 0.1 mol/dm^3 HCl and 0.5 ml 0.01 mol/dm^3 $KMnO_4$. Solution was heated to 80-95°C for 5-10 min. For reducing the excess of permanganate oxalic acid was added. The volume of samples was adjusted to 50 ml with 0.1 mol/dm^3 HCl.

2.5. Determination of pseudo-total zinc concentration

Aqua regia extractable zinc fraction as an adequate fraction for analyzing total-recoverable zinc was determined by extraction in strong acid conditions and analyzed by atomic absorption spectrometry with electrothermal atomization (ETAAS) device (Shimadzu AA-6300, USA) with electrothermal atomizer (Shimadzu GFA-EX7i) using an automatic dispenser (Shimadzu ASC 6100) and background correction method of Smith-Hieftje. Samples were microwave digested by the Multiwave system MW 3000 (Anton Paar GmbH, Austria)

3. Results and discussion

3.1 Characterization of soluble and solid fraction of DANS

Physico-chemical characteristics of DANS are presented in Table 2. On the basis of experimental data we determined cation exchange capacity (CEC) of 25 meq/100g. CEC is commonly used to obtain the soil sorption capacity for heavy metals. Content of the soluble fraction is important for utilization of sludge as soil conditioner. We found, that after one step extraction by 0.9 % NaCl at 22°C, DANS had well sedimentation properties.

Table 2. Chemical and physico-chemical characteristics of DANS studied

DANS characterization			
Soluble fraction[a]		***Solid fraction[b]***	
Proteins[1] [mg/g]	9.6 ± 0.3	Cobalt[5] [µg/kg]	86 ± 3
Carbohydrates[2] [mg/g]	5.1 ± 0.2	Manganese[5] [µg/kg]	163 ± 5
Tiols[3] [µmol/g]	6.1 ± 0.1	Zinc[5] [mg/kg]	649 ± 12
Soluble inorganic salts[4] [mg/dm^3]	<LOD	CEC[6] [meq/100g]	25

[a]extracted by 0.9 % NaCl at 22 °C; [1]determination of soluble proteins (Lowry et al., 1951); [2]determination of carbohydrates by anthrone reagent (Dreywood, 1946); [3] determination of thiols by Ellman's reagent (5,5-dithiobis-(2-nitrobenzoic acid) (Ellman, 1959), [4] determination of soluble inorganic salts by IC (Values of LOD for anions used (µg/dm^3) : fluorides 6, chlorides 7, nitrites 20, bromides 29, sulphates 25, nitrates 24, phosphates 7); [5]concentration of cobalt, manganese and zinc after aqua regia digestion (ETAAS), [6]cation exchange capacity

Concentration of mobile metals in the sewage sludge is important for assessment its hazardous character or using as a soil conditioner and fertilizer (Frišták et al, 2012a). Pseudototal zinc concentration in DANS as an *aqua regia* extractable zinc was 649 ± 12 mg/kg (d.w.). The comparison of obtained extractable fractions of other heavy metals is shown in Table 2. Meers et al (2007) showed that used digestion process can be considered adequate for analysing total-recoverable heavy metals in soils. We suppose that residual zinc that is not released by *aqua regia* digestion is bound to silicate minerals and is considered unimportant for estimating the mobility of metals. Similar results were obtained by Niskavaara et al. (1997).

3.2. Optimization of zinc extraction protocol – RSM application

Generally, sewage sludge as an organic matter-rich material contains larger concentration of heavy metals than the background values found in soil. Application of sludge as soil conditioner can gradually raise the total toxic metal concentration of soil (Smith, et al., 2009). Although, metals present in compost exist in relatively resistant forms, these are more labile in the environment of soil matrix. Zinc is the element which the largest increase in labile forms is reported when sewage sludge is applied to soil, particularly under acidic soil conditions (Planquart et al., 1999). Eneji et al. (2003) found that Zn availability increased during anaerobic digestion of organic wastes. One of the main approaches to study the behaviour of toxic metals in sewage sludge has been to use chemical extraction protocol to remove metals bound in different defined phases (He et al., 1992). Parameters of extraction protocols are variable and it is difficult to find out the optimal conditions. For that reason, a Box-Behnken design under RSM was used to analyze interactive effect of the amount of sludge biomass, concentration of extracting agent and reaction time on total extractable zinc concentration from DANS. The advantage of the RSM method is the minimization of the number of experiments and time needed. A total of 17 runs were used to optimize the extraction process parameters (Table 2). Each of experiment was performed in triplicate.

Using multiple regression analysis, the second order polynomial model which characterizes the relationship between total extractable zinc concentration c_{Zn} from DANS and actual studied variables can be written as given by (Eq. 2). The analysis of variance (Table 3), which is a statistical technique that subdivides the total variation in a set of data into components associated with specific sources of variation to test hypotheses on the parameters of the model, suggests the statistical significance of the model equation, as it is evident from the Fisher`s *F*-value (86.19) with a low probability value (*p*-value <0.0001).

$$\begin{aligned} c_{Zn} = {} & +5.49265 + 4.06566.10^{-3} \times m_{DANS} - 0.13619 \times \\ & c_{HCl} - 2.42866 \times t + 1.01010.10^{-4} \times \\ & m_{DANS} \times 4 + 8.33333.10^{-4} \times m_{DANS} \times t + 0.032828 \times \\ & c_{HCl} \times t - 5.55556.10^{-6} \times m_{DANS^2} + \\ & 1.17335.10^{-3} \times c_{HCl^2} + 0.21875 \times t^2 \end{aligned} \quad (2)$$

A large *F*-value indicates that most of the variation can be explained by a regression equation whereas a low *p*-value indicates that the model is considered to be statistically significant (Myers and Montgomery, 2002). The goodness of fit was also evaluated by the correlation coefficient (R^2) between the model predicted and experimental values. Coefficient R^2 indicates the statistically signification of model used. Both values R^2 and adjusted R^2 are closed to 1 and indicate a high correlation between the observed and the predicted values (Garg et al., 2008).

On the basis of this investigation, the relationship between the independent variables (amount of sludge biomass, concentration of extracting agent and reaction time) and the response (total extractable zinc concentration from DANS) can be explained according to the regression model. c_{Zn} is attributed to the independent variables and only about 1.90 % of the total variation cannot be explained by the model. Regression analysis (Table 3) shows that the square as well as the interaction effects of the independent experimental variables were significant (p<0.0001). The adequacy of quadratic model was tested also through the correlation between calculated and experimental values for total extractable zinc concentration from DANS, which is shown in scattered plot (Fig. 1).

Table 3. BBD matrix, the experimental and predicted values of total extractable Zn concentration c_{Zn} from DANS for three independent variables (A: m_{DANS} (mg); B: c_{HCl} (mmol/dm^3); C: t (h))

Run	***Coded levels***			***Y (c_{Zn})***	
	A	***B***	***C***	***c_{Zn} (exp.) (mg/kg)***	***c_{Zn} (pred.) (mg/kg)***
1	-1	0	+1	5.05	3.25
2	+1	+1	0	19.10	19.37
3	0	+1	-1	12.00	12.00
4	0	0	0	6.00	6.00
5	0	0	0	6.00	6.00
6	+1	0	+1	12.05	12.00
7	0	0	0	6.00	6.00
8	+1	-1	0	1.00	1.12
9	0	-1	-1	5.02	3.12
10	0	+1	+1	21.00	21.87
11	-1	0	-1	1.05	1.00
12	+1	0	-1	5.00	6.75
13	-1	+1	0	10.13	9.87
14	-1	-1	0	1.00	2.87
15	0	0	0	6.08	6.00
16	0	0	0	6.08	6.00
17	0	-1	+1	1.00	0.87

Table 4. Analysis of variance (ANOVA) for Zn extraction from DANS

Source	*Sum of squares*	*DF*	*F value*	*p-value Prob>F*
Model	554.06	9	86.19	<0.0001
A	50.00	1	70.00	<0.0001
B	364.50	1	510.30	<0.0001
C	32.00	1	44.80	0.0003
AB	20.25	1	28.35	0.0011
AC	2.25	1	3.15	0.1192
BC	42.25	1	59.15	0.0001
A^2	5.33	1	7.46	0.0293
B^2	34.80	1	48.72	0.0002
C^2	3.22	1	4.51	0.0713
Lack of fit	5.00	3	0.00	
Pure Error	0.000	4	0.00	
Total	559.06	16		
R^2	0.9754	R^2_{adj}	0.98	

From correlation between predicted $c_{Zn\ pred}$ and experimental $c_{Zn\ exp}$ values of zinc extraction from DANS is evident that the regression model can represent the experimental extraction data well. On the basis of the evaluation of ANOVA outputs, the statistical significance of a quadratic model for the response was confirmed and it can be concluded that the model can be used for further analysis of effect of process variables (Frišták et al., 2012a; Remenárová et al., 2012).

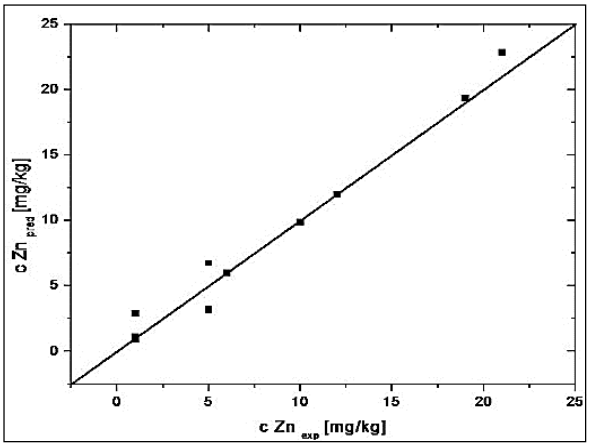

Fig. 1. Correlation between predicted c_{pred} and experimental c_{exp} values of zinc extraction from DANS

As was already mentioned above, the aim of this paper was to optimize the parameters of zinc extraction protocol, namely: the amount of sludge biomass, concentration of extracting agent and reaction time. The statistical significance of each coefficient was determined by *p*-values shown in Table 3. Lower *p*-value indicates a higher significance of the parameter.

Our results demonstrate the statistical significance of linear quadratic coefficients: A (F = 70.00, p <0.0001), B (F = 510.30, p <0.0001) and C (F = 44.80, p = 0.0003), and quadratic coefficients A^2 (F = 7.46, p = 0.0293), B^2 (F = 48.72, p = 0.0002). Other quadratic form C^2 (F = 0.37, p = 0.0713), as well as interaction coefficient AC (F = 3.15, p = 0.1192) not confirmed the statistical significance. For interpretation of the interaction coefficients can be used three-dimensional plots of regression model. Such three-dimensional surfaces can provide useful information about the behaviour of the extraction system within the experimental design, facilitate an examination of the effects of the experimental factors on the responses and contour plots between the factors (Ahmad and Hameed, 2010).

Fig. 2 shows that the extraction process of zinc is strongly influenced by the amount of biomass and concentration of exracting agent. Effect of sludge amount in combination with reaction time was less significant. According to identified relations and predicted values we found out that the extraction process of zinc from DANS has highest efficiency at following parameters: the amount of sludge biomass 550 mg, the concentration of extracting agent (HCl) 100 mmol/dm^3 and contact time 5 h. Correlation among to predicted and experimental data showed ability to reducing the number of total experiments from 39 to 17. Mannan et al. (2007) showed the usefulness of RSM methods and Box-Behnken design for the optimization of process parameters of bioconversion of activated sludge by Penicillium corylophilum.

Response surface method (RSM) appears to be a better tool for the evaluation of difficult studies of multi-component systems than the simple extrapolation from single-component systems in sorption process of heavy metals by dried activated sludge (Remenárová et al., 2012). Zhang and Zhang (2012) showed the application of [*S*,*S*]-EDDS as extraction agent of Zn from mixed sludge with effectivity more than 65%. Authors described equally the possibility of Zn extraction from this type of sewage sludge by EDTA with high efficiency. However extraction of Zn from sewage sludge requires optimization of reaction conditions (Babel and Dacera, 2006). Our paper showed the RSM as useful statistical tool for optimization of Zn extraction process by HCl with comparable removing effect such as extraction by H_3PO_4 published in paper Zhang and Zhang (2012).

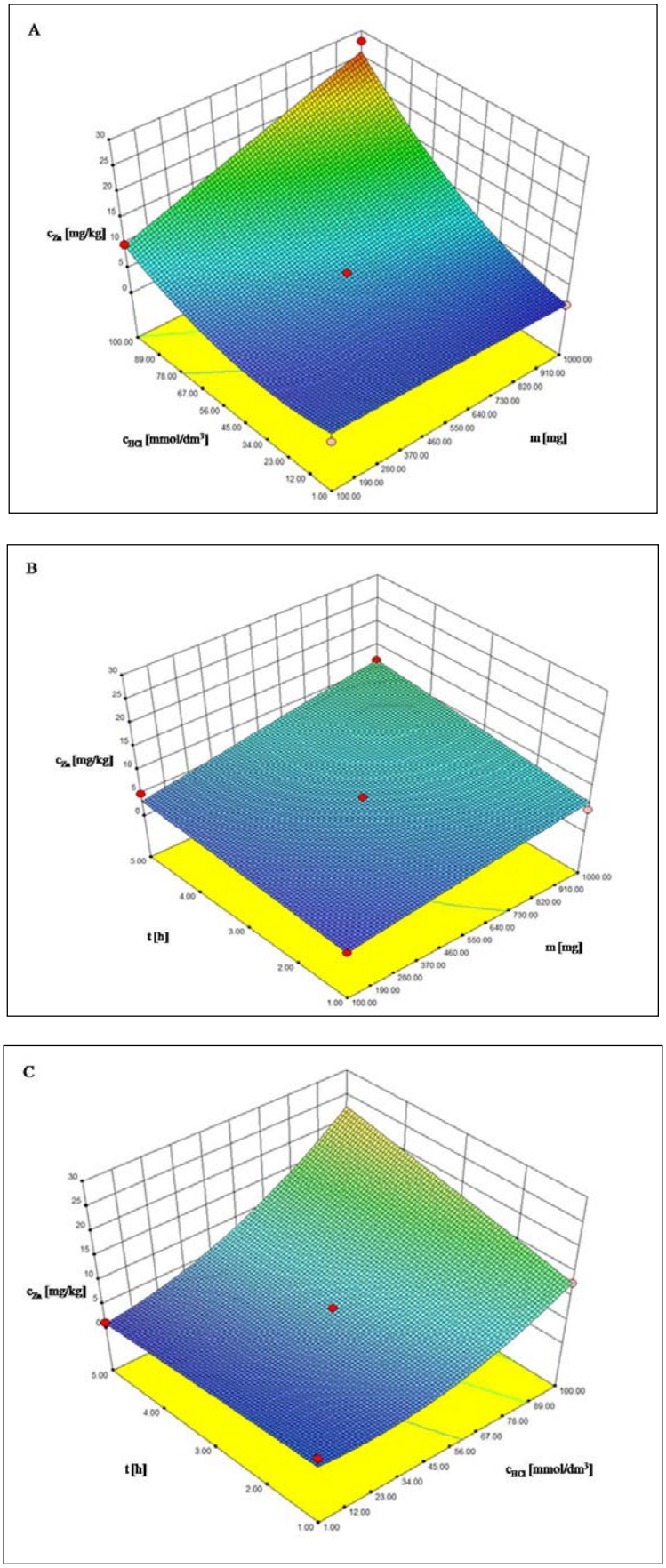

Fig. 2 Three-dimensional surface plots of the combined effect of (A) reaction time (t) and amount of DANS (m); (B) concentration of extracting agent (c_{Zn}) and amount of DANS (m); (C) reaction time (t) and concentration of extracting agent (c_{HCl}) on total extractable zinc concentration (c_{Zn}) from DANS (V_{HCl} = 10 cm^3, 22°C, 150 rpm)

4. Conclusions

The present investigation was carried out to optimize extraction process as a function of amount of DANS biomass, concentration of extracting agent and reaction time using a Box-Behnken design under the Response surface methodology (RSM).

The results demonstrate that interaction effect of reaction time and amount of biomass to Zn extraction is minimal, but in combination with the concentration of HCl significant increase of extractable zinc from DANS was detected. ANOVA analysis as well as 3D surface plots revealed that the maximum metal solubilization was obtained at 0.55 g of DANS, 100 mmol/dm^3 HCl and 5 hour of reaction time. On the basis of results it can be concluded that RSM present an excellent tool in prediction of optimal parameters of extraction protocols and reduces the number of needed experiments from 39 to 17.

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