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Modeling and Optimization of Electrocoagulation Voltage and Water Immersion Time on Heavy Metal Reduction in Fish

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***Abstract*— Fish that are in polluted waters can contain heavy metals reaching 0.62 mg/kg. While the maximum limit of heavy metals in fish is only up to 0.3 mg/kg. Consumption of fish containing high heavy metals can cause poisoning to death. This study aimed to model and optimize electrocoagulation stress and distilled water immersion time on heavy metals content by using response surface methodology (RSM). Several studies have proven the effectiveness of the electrocoagulation process and the process of distilled water immersion to reduce levels of heavy metals in fish. If the two methods are combined, it might increase the effectiveness of reducing heavy metals. This study used two treatment factors, i.e., the distilled water immersion time factor of 30-90 minutes and the electrocoagulation voltage factor of 6-18 volts. From the results of the RSM, the best model to reduce the heavy metals content was a quadratic model. From the control point of heavy metals contamination in tilapia fish of 14.73 ppm, it was found that the highest heavy metals reduction was at the treatment of 35.51 minutes immersion time and 6.24 volts of electrocoagulation voltage. The maximum result of heavy metals reduction in tilapia fish based on predictions was 86.319%. In comparison, the validation test in the actual experiment was 90.21%, so the optimization results can be said to be valid because of the error value (4.5%) was less than 5%.**

***Keywords*— distilled water soaking; electrocoagulation; fish; RSM.**

I. INTRODUCTION

As a result of indiscriminate disposal of wastes, the environment becomes a polluted landfill. One of the hazardous wastes is a heavy metals waste [1]. Heavy metals include Zn, Cu, Fe, Co, Mn, Se Hg, Cd, Pb, Sn, Cr, As [2]. One of the heavy metals that are often found as waste and are dangerous is Lead (Pb) [3]. Heavy metals pollution in the world has spread to waters such as rivers and seas so that Pb heavy metals spread to the biota like fish [4], [5]. The impact, if humans consume the fish, can cause some negative impacts, i.e., damage to the nervous system, damage to the blood formation system, kidney damage, cancer risk, and even can cause death [6]. If heavy metals have contaminated the fish, the fish is no longer suitable for consumption [7].

To overcome the problem of heavy metals pollution, effective treatment of heavy metals content in fish is needed [8]. Some methods that can be used in reducing levels of heavy metals are immersion with tamarind acid, distilled water immersion, conjoined orange filtrate immersion, chitosan adsorbent immersion, lime juice immersion, and electrocoagulation [9-12]. However, some methods such as immersion with acid solutions, conjoined orange filtrate immersion, and the use of chitosan adsorbents have the disadvantage of influencing the taste and the quality of the fish product [13]. In some studies, the immersion of distilled water is mostly used as a comparison between other submersions, such as previous studies that used lime juice to reduce levels of heavy metal Pb, from these studies with the immersion of heavy metal distilled water can drop by 63.42% [14]. Electrocoagulation is a method that is widely used to precipitate wastes, such as industrial, laboratory, and household wastes, which contain heavy metals in them. By using the electrocoagulation method with aluminum, electrodes can reduce metal content by 50-99% in electroplating wastewater [15]. The mechanism of electrocoagulation in removing heavy metals in fish is by pulling Pb heavy metal content in fish through immersion of distilled water and then deposited into flocks, which will then settle to the bottom of the electrocoagulation bath. The advantages and disadvantages of the two methods are that the fish from immersion still has the same taste; in other words, soaking distilled water does not affect the taste of the fish soaked. Still, with immersion, the distilled water results in a decrease in heavy metal content are relatively smaller than the acid immersion method.

Meanwhile, if only using electrocoagulation alone, the Pb content in fish cannot come out perfectly. For the electrocoagulation process, aluminum electrodes are used. The combination of electrocoagulation methods with water immersion is an innovation that has the potential to reduce the heavy metal content in fish effectively. The combination of these methods from preliminary experiments can effectively reduce heavy metals in fish. But in the initial research, optimal points of water immersion time and electrocoagulation voltage to maximize the reduction of the heavy metals in fish is still unknown.

Response Surface Methodology (RSM) is used for modeling and optimization in this study. RSM has proven to be very effective in optimizing some biological problems [16-18]. This research aims to model and optimize the electrocoagulation voltage and the immersion time of distilled water on heavy metal reduction in fish. RSM is commonly used to determine the optimal conditions of a response that is influenced by interactions between variables [19]. RSM can produce a mathematical model of the relationship between independent variables on the response. The form of this relationship is generally a polynomial that is first-order or second-order, but the second-order model can optimize responses significantly compared to first order.

II. Materials and Methods

This research was conducted at the Mechatronics Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya, Indonesia, and in testing heavy metal content was conducted at the Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Indonesia. The tools used in this study are as follows:

- Multimeter to measure the voltage in the electrocoagulation process.
- Digital scales to measure the mass of fish and Pb acetate powder.
- LM2596 to regulate the size of the voltage that comes out in the electrocoagulation process
- Stopwatch for adjusting the time of water immersion
- Design Expert 11 as data processing software
- A 24V power supply as a source of voltage for the electrocoagulation process
- Jumper cables to connect electrodes to the power supply
- Container box with a size of 20×10×12 cm as a place to put the fish for the electrocoagulation process
- Electrodes with a size of 12×0.1×4 cm as cathodes and anodes.

While the materials used in this study are as follows:

- distilled water as immersion media.
- tilapia fish (*Oreochromis niloticus*) of 13 samples for training and 5 samples for validation
- Pb acetate powder as heavy metal contaminants.

This study was divided into several stages i.e. the tool design stage, the fish quarantine stage with Pb contaminants, the RSM central composite design (CCD) stage for modeling and optimization, and the validation process. The heavy metals reduction by immersion process was using distilled water of 20 L. Heavy metals reduction device consisted of a combination of two methods i.e. water immersion device and electrocoagulation device. The container box as the water immersion device was made of acrylic material with a thickness of 3 mm. The immersion box was filled with distilled water of approximately 2 L. The electrodes were assembled into the immersion box for the electrocoagulation process. The electrocoagulation design can be seen in Figure 1. The electrodes used were aluminum with dimensions of 4×9 cm with a plate thickness of approximately 1 mm, and between the cathode and anode, there was a distance of 2 cm. The use of the 2 cm distance was based on the optimum result of the preliminary research. The copper conductor was used as a conductor of electricity to electrodes because copper is a good conductor of electricity. The power source was connected to the electrodes in parallel so that the voltage produced by each electrode was the same. To vary the voltage value, a module was used to adjust the output voltage of the power supply. The number of electrodes used were eight pairs. The use of 8 pairs of electrodes was based on the optimum result of the preliminary research. In the middle part, there was an empty electrode circuit or space which was used to place fish samples containing heavy metals. The electrodes were initially attached to the wire in the electrocoagulation container. Then the distilled water is put into the 2 L electrocoagulation container. Electrodes that have been installed are then connected to wires. Furthermore, the voltage in the power supply is set according to the desired voltage. Finally, the prepared sample is then put into an electrocoagulation container together with a cable connected to the power supply.

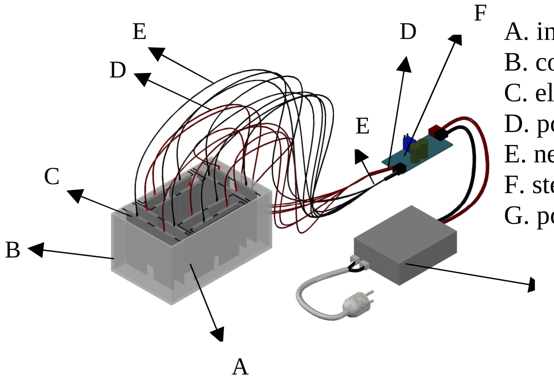

Fig. 1. Electrocoagulation design

The fish quarantine process begins with mixing Pb acetate with water at 60 ppm. Then the water that has been mixed with Pb is allowed to stand for 60 minutes. After that, the fish were transferred to the water with a mixture of Pb acetate for 24 hours. In the quarantine process, Pb contamination is influenced by quarantine time and Pb concentration in water, where Pb heavy metal contamination in fish increases following the Pb concentration in water. The process of accumulation of heavy metals Pb in fish bodies is influenced by the length of time and the amount of Pb contaminants in water. In this quarantine process, it is expected to obtain fish that contain heavy metals, not only on the surface but can reach the fish meat. Resulting in heavy metal contamination fish as a control point of 14.73 ppm.

This research was conducted by the RSM method with a model using CCD. With the factors used as follows: X1 = water immersion time for 30-90 (minutes); X2 = electrocoagulation voltage of 6-18 volts. The use of immersion time factor and electrocoagulation voltage was based on previous studies using processing time for 60 minutes and the voltage used were 12 volt with the number of 8 pairs of electrodes, which was the most effective in reducing heavy metal content. After obtaining X1 and X2 as the minimum and maximum points of Table 1. The next step was data analysis and optimization using the Design Expert 11 software. From the results of CCD, obtained 4 factorial points, 4 axial points, and 5 center points. The 4 factorial points consist of coordinates 1 and -1, with coordinate 1 as the maximum value of the X1/X2 factor, and coordinate -1 as the minimum value of the X1/X2 factor. For 4 axial points consisted of coordinates α, 0, and -α. The α value used 2 factors i.e. the value α = 1.414, with coordinates 1414 at X1 102.4 and X2 20.49 and -α = -1.414 with coordinates -1.414 at X1 17.6 and X2 3.51. For the center point, using 5 points with centers X1 60 and X2 12.

The use of 5 centers was expected to predict the determination of the optimal point covering all areas, both edge and center, and expected results from the optimal point in the middle. This central point was needed to support the stability of the variance of the estimated response value. So in total, there were eight treatments with 13 observations. From these 13 observations, the response variable was then sought, which was the value of heavy metal content, so that the optimum immersion time and electrocoagulation voltage was obtained in the process of minimizing heavy metal content.

TABLE I
CCD CONSTRAIN DATA

	Name	Units	Low	High	-alpha	+alpha
A [Numeric]	Immersion time	minute	30	90	17.57	102.43
B [Numeric]	Voltage	volt	6	18	3.51	20.48

Heavy metal content testing was carried out using an atomic absorption spectrophotometer. The sample tested was the gill part of the fish. This is because the part that is vulnerable to heavy metal contamination is the gills. Because in addition to functioning for breathing, the gill function is also used for filtering food consumed by fish. Heavy metal content testing uses a spectrophotometer using two methods i.e. wet destruction and dry destruction. In this research, the wet destruction method was used. For wet destruction in heavy metal content testing, there were several steps, including setting the atomic absorption spectrophotometer, making a standard calibration curve for lead, analyzing the lead content, and finally, the data analysis.

In short, these steps if explained briefly, initially the gill sample was dried and then mashed. After smoothing, as much as 5 g of the sample was mixed with 30 mL of concentrated HNO_3 in small increments and then heated at 85 ºC for 8 hours in a fume hood. During the heating process, the solution was stirred continuously until it was evenly mixed, and the sample was destroyed, and before the destruction process was stopped, add 10 mL of 30% H_2O_2 by dripping until the solution was clear. After that, it was cooled to room temperature. A day later, the sample solution was filtered with Whatman filter paper no. 42. After that, the measurement of Pb metal content at a wavelength of 217.0 nm. Finally, the data analysis was the data obtained from the spectrophotometer reading and then analyzed by the linearity, sensitivity, accuracy, and detection limit and quantitation limit and performed twice (Duplo).

III. RESULTS AND DISCUSSION

From the research that had been done, the data in Table 2 shows, from the measurements of heavy metal content showed that the lowest value was obtained in column 12 with 60 minutes immersion, and 12-volt voltage used was obtained 2.24 ppm metal content. However, it cannot be said of the best composition, because of the two parameters did not yet know which was the most influential in the process of minimizing heavy metal content. However, if the time used was 60 minutes, the heavy metal reading was mostly low, and if the voltage used was 12 volts, the reading of heavy metal content was also low. It means if the voltage and time used were more than the middle point was used, the metal content read will be a lot, and if the time and voltage used were too low, then the heavy metal read was also high. There were two processes, i.e., the entry of water content in the immersion process and the release of Pb heavy metal content, and from the process most likely the distilled water has contained contaminants or Pb heavy metal ions. Hence, Pb had previously come out of the fish body again to enter the body of the fish due to the immersion process is too long.

TABLE II
EXPERIMENTAL DESIGN AND RESULTS DATA

Std	*Run*	Factor 1 A: immersion time (minute)	Factor 2 B: Voltage (volt)	Response 1 Heavy metals reduction (%)
1	4	30	6	82.21
2	6	90	6	39.92
3	13	30	18	16.43
4	3	90	18	24.71
5	10	17.57	12	72.51
6	9	102.43	12	72.71
7	7	60	3.51	77.39
8	5	60	20.49	60.76
9	2	60	12	68.09
10	11	60	12	83.77
11	1	60	12	74.95
12	12	60	12	84.79
13	8	60	12	84.66

Analysis of statistical models was divided into several, i.e., Sequential Model Sum of Squares, Lack of Fit, and Statistical Summary Models. In the Sequential Model Sum of Squares data analysis, the p-value must be less than 5% ($p < 0.05$). From the selection of Sequential Model Sum of Square, it was suggested that the quadratic model, as shown in Table 3. However, from the p-value, all p-value values from the linear model, the 2-factor model (2FI), the quadratic model, and the cubic model, shows the p-value was more than 5% ($p > 0.05$). It means that all models did not meet the requirements, where the p-value must be less than 5% ($p < 0.05$). However, because the p-value close to 5% was a quadratic model, the program suggested using a quadratic model compared to other models.

TABLE III
MODEL SELECTION BASED ON SEQUENTIAL MODEL SUM OF SQUARES

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	54652.34	1	54652.34			
Linear vs Mean	1507.44	2	753.72	1.53	0.263	
2FI vs Linear	639.33	1	639.33	1.34	0.276	
Quadratic vs 2FI	1700.47	2	850.23	2.30	0.171	Suggested
Cubic vs Quadratic	559.87	2	279.94	0.69	0.543	Aliased
Residual	2026.43	5	405.29			
Total	61085.88	13	4698.91			

Data analysis based on the Lack of Fit Test aimed to compare residual errors with Pure Error. In Table 4, the p-value of all models of the linear model, the 2-factor model (2FI), the quadratic model, and the cubic model showed a p-value of less than 5% ($p < 0.05$). This means that all models did not meet the requirements, where the p-value must be more than 5% ($p > 0.05$). However, the p-value approaching 5% was a quadratic model, so the program suggests using a quadratic model compared to other models.

TABLE IV
MODEL SELECTION BASED ON LACK OF FIT TESTS

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	4702.67	6	783.78	14.03	0.012	
2FI	4063.34	5	812.67	14.55	0.011	
Quadratic	2362.87	3	787.62	14.10	0.014	Suggested
Cubic	1803.00	1	1803.00	32.28	0.005	Aliased
Pure Error	223.43	4	55.86			

Finally, for model selection using the Summary Statistical Model, it was suggested a quadratic model. This was indicated by the information suggested in Table 5. For this method, the actual determination of the model was based on a low standard deviation value, a high R^2 value, and a low PRESS value. Determination of the right model in this Summary Statistical Model is the lowest standard deviation parameters, the highest R^2, the highest Adjusted R^2, the highest Predicted R^2, and the lowest PRESS.

TABLE V
MODEL SELECTION BASED ON MODEL SUMMARY STATISTIC

Source	Std. Dev.	R²	Adjusted R²	Predicted R²	PRESS	
Linear	22.19	0.23	0.08	-0.49	9568.85	
2FI	21.82	0.33	0.11	-1.63	16903.46	
Quadratic	19.22	0.60	0.31	-1.67	17151.77	Sugges-ted
Cubic	20.13	0.69	0.24	-16.99	1.157E+005	Aliased

From Table 5 the lowest standard deviation values were found in the quadratic model with a standard deviation value of 19.22. The highest value for R^2 was in the cubic model, with a value of 68.50%. However, because in this experiment was using the design of 2 variables, the cubic model was not used, so the highest value in the R^2 value was in the quadratic model, with a value of 59.80%. Furthermore, the highest adjusted R^2 was in the quadratic model, with a value of 0.3109. The highest predicted R^2 value was in the linear model with a value of -0.4873. The lowest PRESS value was in the linear model, with a PRESS value of 9568.85. Even though the last 2 parameters showed a linear model, it was still recommended a quadratic model.

ANOVA analysis results in Table 6, the quadratic model was not significant with an F-value of 2.08 and a P-value of 0.1829 which indicated that there was an 18.29% chance that the F-value can be that high due to noise. The immersion time (A) and the voltage used (B) were also not significant to the response because they have P-values of 0.5547 and 0.0960, respectively. The interaction of two factors (AB) immersion time (A^2) and the voltage used (B^2) also had a P-value of more than 5%, i.e., 0.2298; 0.1809; and 0.1276. Table 6 showed that the Lack of Fit model was not real, with a P-value of 0.0136. This value had fulfilled the requirements of P <5% so that significant information was listed. The RSM suggested that it used the quadratic model.

TABLE VI
ANOVA ANALYSIS

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3847.24	5	769.45	2.08	0.183	not signi-ficant
A-Immer-sion	142.19	1	142.19	0.38	0.555	
B-Voltage	1365.25	1	1365.25	3.70	0.097	
AB	639.33	1	639.33	1.73	0.230	
A²	815.51	1	815.51	2.21	0.181	
B²	1103.50	1	1103.50	2.99	0.128	
Residual	2586.30	7	369.47			
Lack of Fit	2362.87	3	787.62	14.10	0.017	Signi-ficant
Pure Error	223.43	4	55.86			
Cor Total	6433.54	12				
Std. Dev.		19.22		R²		0.60
Mean		64.84		Adjusted R²		0.31
C.V. %		29.65		Predicted R²		-1.67
PRESS		17151.77		Adeq Precision		3.94

Based on the analysis results obtained second-order polynomial equations in the form of coded variables and actual variables. Equations in the form of coded variables:

$$Y_{heavy\ metals\ reduction} = 79.25 - 4.22x_1 - 13.06x_2 + 12.64x_1x_2 - 10.83{x_1}^2 - 12.59{x_2}^2$$

Where x_1 is the time of immersion in distilled water, and x_2 is the voltage used. Whereas the order polynomial equations of the two forms of actual variables were:

$$Y_{heavy\ metals\ reduction} = 70.692 + 0.460T + 2.005V + 0.070TV - 0.012T^2 - 0.349V^2$$

Where T is the immersion time (minutes), and V is the voltage (volt).

The accuracy of the model can be seen from the distribution of actual and predicted values in Figure 2. From the distribution of predicted and actual points, it can be concluded that the results of the distribution of actual and predictive values are less good because the points move away from the diagonal line of the graph. Where these points are actual values and diagonal lines are predictive values with linear models.

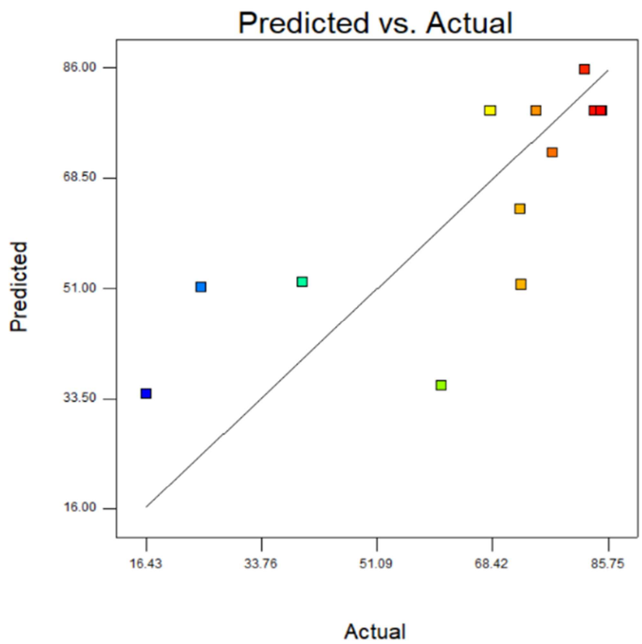

Fig. 2. Distribution of actual and predicted value

From Figure 3, there was a relationship between the immersion time and the voltage used. Therefore, it can be concluded that the graph had a significant effect on the minimization of heavy metal content in fish. That was because the 3D graph forms a quadratic graph model forming a valley, where if the graph shows a quadratic shape, then there was a valley area of the graph, and the valley area later got the minimum metal content in fish. However, the graph still had an optimization area or an area that can be searched for its optimum point again, i.e., the blue area in Figure 3. This was because the extent of the optimization area on the graph affected the number of solutions obtained. The combination of the immersion distilled water and the voltage used has a real impact, and from these data, it can be concluded that the longer the immersion time and the voltage used, the greater the contamination and when the immersion time was more than 30 minutes and less than 60 minutes and the voltage used was less from a 12-volt reduction in heavy metal content the greater. This was because fish containing contaminants at the beginning of the process, the fish undergoes a diffusion process, Pb contaminants came out and are directly processed by the electrodes. However, when the immersion process was too long, then the Pb that had come out can come back because the fish was too long dead. In a submerged position then the fish will absorb a lot of water, and because the water in it contains Pb contaminants, it allowed Pb to come in with the water. For the case, the greater the voltage, the smaller the weight of the heavy metal; this was because during the electrocoagulation process, the higher the applied voltage, the longer the time required for floc deposition. Because the electrocoagulation and immersion processes occur together, heavy metal contaminants will be reabsorbed into the body of the fish.

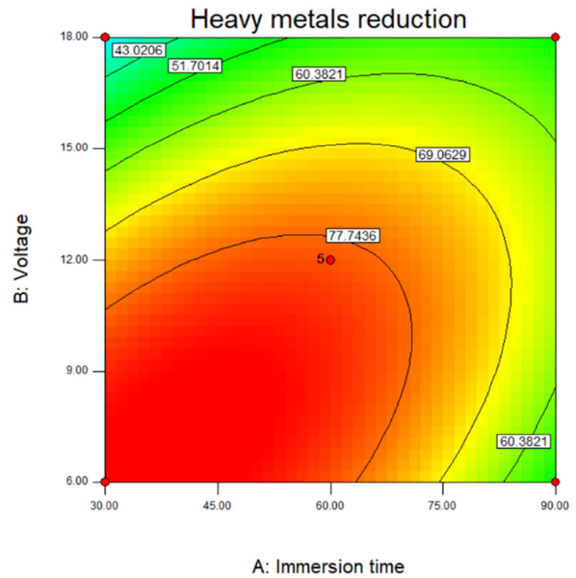

Fig. 3. Contour plot of heavy metals reduction

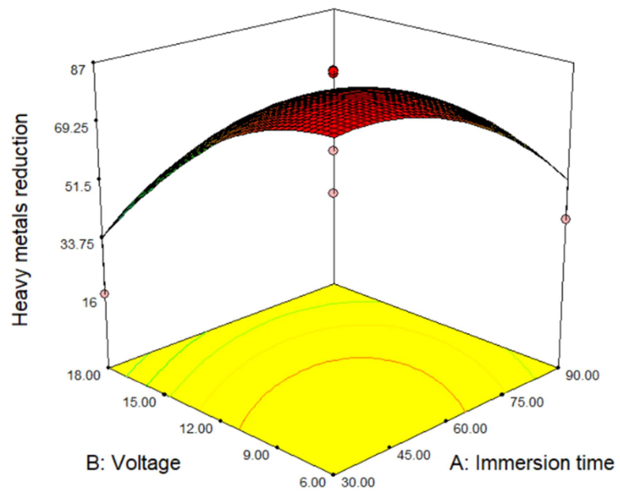

Fig. 4. Graphic of heavy metals reduction in 3D

Optimization of a heavy metal response aims at determining the best treatment value. Based on Table 7 it can be seen that the optimum immersion time occurred at 35.51 minutes, and the optimum voltage was 6.24 volts, and the response of heavy metal content was 86.319%. In this optimization, the desirability point was obtained for 1. The desirability value is used to determine the accuracy of the optimal solution results with a range of values from 0 to 1, where the value 1 indicates that the perfect case response while 0 indicates the response must be discarded. So, with a desirability value of 1 can indicate the accuracy of the optimum point response in this study by 100%. And from the desirability graph in Figure 4, there were green to reddish areas. The redder the better the desirability value.

The desirability area was in the red area which indicates that the point was very good.

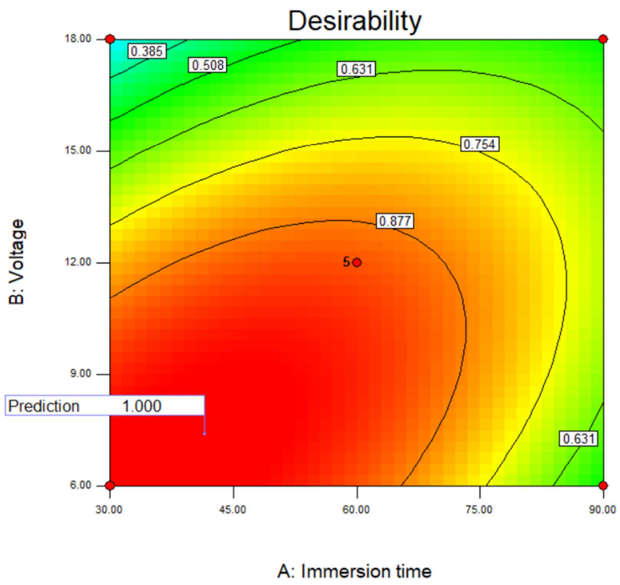

Fig. 5. Contour plot desirability

TABLE VII
SELECTED OPTIMUM POINT

Immersion time (minute)	Voltage (volt)	Heavy metals reduction (%)	Desirability
35.51	6.24	86.319	1.000

The optimal immersion time was 35.51 minutes because, at that time, the immersion process did not run so fast or long. If the immersion process was too fast, then the heavy metals in the fish will not be reduced to the maximum, and if the soaking was too long, then the heavy metals that have come out can also be reabsorbed in the body of the fish. The cause of re-absorption of heavy metals due to damage to tissue in the body of the fish at the time of death of the fish caused an increase in the activity of proteolytic enzymes. As a result, the fish were easily decomposed, and from the process, the fish become easily contaminated, so that fish that were soaked for too long can easily be re-contaminated by heavy metals, causing immersion that was too long to be less effective.

The optimal voltage was 6.24 volts because by using this voltage, the electrodes were more effective in absorbing heavy metals in the body of the fish. After all, if the Pb binding process becomes less effective and if it was too large, then the distilled water becomes saturated so that the distilled water becomes less effective in drawing heavy metals on the body of a fish. Distilled water was too saturated due to too fast the reduction and oxidation of the electrodes that cause a lot of the formation of ions and compounds in the distilled water.

Validation of the optimum model was done by comparing the percentage of predictive response results with the percentage of response results from research Table 8. The percentage of predicted and actual results obtained from the percentage of decline with a control point of 14.73 ppm. The percentage of the predicted value and the percentage of the actual value obtained a difference of 4.5%.

TABLE VIII
OPTIMUM VALIDATION RESULT

Variable	Optimum point	Heavy metals reduction	
		Prediction (%)	Actual (%)
Immersion time (minute)	35.51	86.319	90.21
Voltage (volt)	6.24		

IV. CONCLUSION

The optimum results obtained from the maximation of heavy metal reduction using Response Surface Methodology (RSM) was 86.319% using an immersion time of 35.51 minutes and an electrocoagulation voltage of 6.24 volts. The response resulting from the minimization of heavy metal content was the quadratic model. The actual value of the validation results showed a heavy metal reduction of 90.21%. With a control point of 14.73 ppm obtained a percentage of the predictive value of 86.319%. From the comparison of the percentage of the predicted value and the percentage of the actual value obtained a difference of 4.5%.

REFERENCES

[1] Tian, K, Wu, Q, Liu, P, Hu, W, Huang, B, Shi, B, Zhou, Y, Kwon, B, Choi, K, Ryu, J, Khim, J S, Wang, T. “Ecological risk assessment of heavy metals in sediments and water from the coastal areas of the Bohai Sea and the Yellow Sea”, *Environment International*, vol. 136, pp. 105512, 2020.

[2] Yu, R, Hu, G, Lin, C, Yang, Q, Zhang, C, Wang, X. “Contamination of heavy metals and isotopic tracing of Pb in intertidal surface sediments of Jinjiang River Estuary, SE China”, *Applied Geochemistry*, vol. 83, pp. 41-49, 2017.

[3] Conversa, G, Miedico, O, Chiaravalle, A E, Elia, A. “Heavy metal contents in green spears of asparagus (Asparagus officinalis L.) grown in Southern Italy: Variability among farms, genotypes and effect of soil mycorrhizal inoculation.”, *Scientia Horticulturae*, vol. 256, pp. 108559, 2019.

[4] Milenkovic, B, Stajic, J M, Stojic, N, Pucarevic, M, Strbac, S. “Evaluation of heavy metals and radionuclides in fish and seafood products”, *Chemosphere*, vol. 229, pp. 324-331, 2019.

[5] Aytekin, T, Kargin, D, Cogun, H Y, Temiz, O, Varkal, H S, Kargin, F. “Accumulation and health risk assessment of heavy metals in tissues of the shrimp and fish species from the Yumurtalik coast of Iskenderun Gulf, Turkey”, *Heliyon*, vol. 5, 02131, 2019.

[6] Rahman, M S, Hossain, M S, Ahmed, M K, Akhter, S, Jolly, Y N, Kabir, M J, Choudhury, T R. “Assessment of heavy metals contamination in selected tropical marine fish species in Bangladesh and their impact on human health”, *Environmental Nanotechnology, Monitoring & Management*, vol. 11, 100210, 2019.

[7] Luczynska, J, Paszczyk, B, Luczynski, M J. “Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health”, *Ecotoxicology and Environmental Safety*, vol. 153, pp. 60-67, 2018.

[8] Vareda, J P, Valente, A J M, Duraes, L. “Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review”, *Journal of Environmental Management*, vol 246, pp. 101-118, 2019.

[9] Doggaz, A, Attour, A, Mostefa, M L P, Tlili, M, Lapicque, F. “Removal of heavy metals by electrocoagulation from hydrogenocarbonate-containing waters: Compared cases of divalent iron and zinc cations”, *Journal of Water Process Engineering*, vol. 29, 100796, 2019.

[10] Kim, T, Kim, T K, Zoh, K D. “Removal mechanism of heavy metal (Cu, Ni, Zn, and Cr) in the presence of cyanide during electrocoagulation using Fe and Al electrodes”, *Journal of Water Process Engineering*, vol. 33, 101109, 2020.

[11] Vakili, M, Deng, S, Cagnetta, G, Wang, W, Meng, P, Liu, D, Yu, G. "Regeneration of chitosan-based adsorbents used in heavy metal adsorption: A review", *Separation and Purification Technology,* vol. 224, pp. 373-387, 2019.

[12] Thilagar, Gobinath, Samuthirapandian, Ravichandran. "Chitosan from crustacean shell waste and its protective role against lead toxicity in Oreochromis mossambicus", *Toxicology Reports*, vol. 7, pp. 296-303, 2020.

[13] Bonilla, F, Chouljenko, A, Lin, A, Young, B M, Goribidanur, T S, Blake, J C, Bechtel, P T, Sathivel, S. "Chitosan and water-soluble chitosan effects on refrigerated catfish fillet quality", *Food Bioscience,* vol. 31, 100426, 2019.

[14] Xu, X, Yang, Y, Wang, G, Zhang, S, Cheng, Z, Li, T, Yang, Z, Xian, J, Yang, Y, Zhou, W. "Removal of heavy metals from industrial sludge with new plant–based washing agents", *Chemosphere*, vol. 246, 125816, 2020.

[15] Dura, Am Brslin, C B. "Electrocoagulation using aluminum anodes activated with Mg, In and Zn alloying elements", *Journal of Hazardous Materials,* vol. 366, pp. 39-45, 2019.

[16] Nejad, Z G, Borghei, S M, Yaghmaei, S, Zadeh, A H. "Developing a new approach for (biological) optimal control problems: Application to optimization of laccase production with a comparison between response surface methodology and novel geometric procedure", *Mathematical Biosciences,* vol. 309, pp. 23-33, 2019.

[17] Maniyam, M N, Hari, M, Yaacob, N S. "Enhanced methylene blue decolourization by Rhodococcus strain UCC 0003 grown in banana peel agricultural waste through response surface methodology", *Biocatalysis and Agricultural Biotechnology,* vol 23, 101486, 2020.

[18] Asadu, C O, Egbuna, S O, Chime, T O, Eze, C N, Kevin, D, Mbah, G O, Ezema, A C. "Survey on solid wastes management by composting: Optimization of key process parameters for biofertilizer synthesis from agro wastes using response surface methodology (RSM)", *Artificial Intelligence in Agriculture,* vol. 3, pp. 52-61, 2019.

[19] Roy, S, Manna, S, Sengupta, S, Ganguli, A, Goswami, S, Das, P. "Comparative assessment on defluorination of waste water using chemical and bio-reduced graphene oxide: Batch, thermodynamic, kinetics and optimization using response surface methodology and artificial neural network", *Process Safety and Environmental Protection*, vol 111, pp. 221-231, 2017.