

HEAVY METAL BIOSORPTION BY POLYPHENOL-FREE BANANA PEEL POWDER

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ABSTRACT

Approach to efficiently manage banana processing waste was proposed by extracting phenolic compound from preserved banana peel powder (PBPP) and then utilizing retentate of this extraction to remove lead and copper from water. The parameters for maximum removal of lead and copper using banana peel retentate (BPR) were optimized in batch biosorption system via response surface methodology (Box-Wilson Design). The optimum values found include: pH, contact time, metal ion concentration and temperature which were 6, 20 min, 20 ppm and 20 °C for both metals, and bio-sorbent dose of 3 and 1 g/L for lead and copper, respectively. At optimized conditions, reduction of lead and copper (87.30% and 67.50%, respectively) by BPR was lower than by PBPP without phenolic extraction (94.20% and 73.00%). Furthermore, one month storage of vacuum-oven dried banana peel at room temperature showed no significant effect on the yield of polyphenols as well as on the biosorption capacity of banana peel.

Keywords: Banana peel, drying, polyphenols, bio-sorbent, heavy metals.

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INTRODUCTION

Banana (*Musa spp.*) is produced worldwide and play socioeconomic role in developing countries as it is mainly cultivated by small landholder farmers (Mapongmetsem *et al.*, 2012). It is mainly cultivated in Pakistan, India, Indonesia, Bangladesh, Vietnam, Pacific Island countries, Philippines, Thailand, and also has an important position in agricultural economy of Latin America and the Caribbean (FAO, 2017). It is generally consumed as fresh fruit and is also processed as part of tropical beverages and as ingredient in various food products (Mohapatra *et al.*, 2011). The consumption of this high amount of production generally generates large quantities of banana peel waste which counts about 40% of the total production. This waste could be used for production of ethanol (Parthiban *et al.*, 2011), methane (Odedina *et al.*, 2017), feed for livestock (Katongole *et al.*, 2011), as source of bioactive compounds (Fidrianny *et al.*, 2014), or as adsorbents for water purification (Borhan *et al.*, 2014).

The concept of dual-purpose utilization of plant waste is not very new and found its root from the work of Shaikh *et al.* (1992) who used linseed stalk first for the extraction of fiber and then developing paper from residual woody straws of extraction. The idea was further extended to develop a plant crop for more than one objective. For instance, duckweed was utilized for wastewater purification from agricultural drains and after

harvesting it as an economical animal fodder with high protein contents (Nassar *et al.*, 2015). The development of dual purpose wheat-cultivars is a potential ideotype to use them as high grain yield and then utilizing their straw for feed and fuel (Townsend *et al.*, 2017).

Unfortunately, no work is available in literature on dual-purpose utilization of banana peel. The extraction of bioactive compounds (polyphenols) from banana peel could be the novel idea for value addition in waste management. There is huge attention in employment of polyphenols as useful ingredients in the development of functional foods because they assure cell constituents, guard against oxidative injury and lower the risk of cardiovascular diseases and cancer (Agama-Acevedo *et al.*, 2016). The extraction of bioactive components generally leaves non-extractable complex carbohydrates and polymeric polyphenols (Pérez-Jiménez *et al.*, 2013) which could be potentially used as bio-sorbent for water purification to remove heavy metals (Vilardi *et al.*, 2017). Heavy metals which are injurious to human health specially lead (Pb) and copper (Cu) cause some chronic types of diseases such as cancer, cardiovascular disease, adverse reproductive outcomes, teeth decay and neurological diseases (Gudzovskij *et al.*, 2004). Among all the purification technologies, adsorption using agricultural waste is better technology for the treatment of polluted water because it is cheap, convenient, operated easily and simple in design (Michalak *et al.*, 2013). Among agricultural waste, the banana peel is potential material for the biosorption of heavy metals as it

contains about 75% cellulose (Oyewo *et al.*, 2016). In this regard, the major challenge faced by the industry is to provide these types of powder with longer shelf life. The vacuum oven drying could be the best method to get the banana peel powder with maximum retention of physicochemical and antioxidant properties (Vu *et al.*, 2017). Thus, aim of the present study was the extraction of polyphenols from preserved banana peel and then response surface statistical optimization of adsorption process to remove heavy metals using banana peel byproduct of polyphenol extraction process.

MATERIALS AND METHODS

Procurement of materials: All the chemicals were of analytical grade from Dukson, Korea and standards were procured from Sigma-Aldrich. Fresh bananas (*Musa acuminata* L. Cavendish Dwarf) were procured from the local market of Faisalabad, Pakistan and graded based on their maturity, while spoiled or damaged fruits were discarded.

Preparation of sample: The banana peel was separated from fruit carefully and dried in a vacuum oven at 60 ± 5 °C under vacuum pressure of 69 kPa until constant weight. The dried banana peel was ground into fine powder in grinder (Panasonic, Japan Model MJ-W176P). The powder was sieved using a 60 mm mesh size sieves and finally, the dried banana peel powder (DBPP) was stored in air tight plastic bags at room temperature (25 ± 2 °C) for one month. After a month, the preserved banana peel powder (PBPP) was stored at freezing temperature till further use.

Extraction of polyphenols: Ultrasound-assisted extraction was performed for maximum extraction of polyphenols from processed banana peel powders by following method of Khan *et al.* (2010) with little modification. For each, a 10 g of powder was added to 200 mL of 70% ethanol and sonicated at 50% amplitude and 750W power for 15 min using ultrasonic equipment (VCX 750, Sonics, USA). The extract was analyzed for total phenolic contents and antioxidant activity while the banana peel retentate (BPR) after filtration was dried in a hot air oven at 100 ± 5 °C for further use in water purification.

Analysis of extract

Total phenolic content: The total phenolic contents (TPC) determination was carried out using a modified Folin–Ciocalteu method given by Huma *et al.* (2018). A 100 µL sample or standard, 1.25 mL UV-grade methanol and 150 µL diluted Folin were taken in a 5 mL test tube and given stay for 5 min. Then 1.5 mL sodium carbonate 6% was added in the mixture and kept in dark for one hour. SpecCord 200 plus UV-visible Spectrophotometer

(Analytik-jena, Germany) was used to measure the absorbance of mixture at 765 nm. Gallic acid was used as standard for calibration and the results were expressed as mg gallic acid equivalent per gram of dry matter (mg GAE/g DM).

Free radical scavenging activity: The free radical scavenging activity (FRSA) of the extracts was estimated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as a standard free radical model and a method adopted from Khan *et al.* (2010) with slight modifications. Sample (2 mL) was taken in a test tube containing diluted DPPH (0.025 g in 100 mL UV-grade methanol). The absorbance was measured at 517 nm with UV-visible Spectrophotometer. The total FRSA of each extract was expressed as the percentage of DPPH reduced and was calculated by the following equation:

$$\text{FRSA (\%)} = 100 \times (A_i - A_f)/A_i$$

The initial absorbance (A_i) and final absorbance (A_f) are the absorbance values of DPPH at time zero and after 60 min, respectively.

Biosorption of heavy metals: Biosorption experiments were performed using BPR in batch system to get reliable and consistent results (Castro *et al.*, 2011). All experiments were carried out according to Box-Wilson design to optimize conditions needed for the maximal adsorption (see section 2.7). For this purpose, a 100 mL of water having 20 ppm to 60 ppm final concentration of lead and copper was taken in conical flask containing 2 to 6 g of alkaline-treated BPR. The pH was adjusted from 2 to 6 by using 0.1 M HCl/NaOH. The flask was placed on magnetic stirrer with constant speed of 200 rpm for 20 to 60 min. The slurry in flask was filtered by using Whatman filter paper 40. Atomic absorption spectroscopy was used to study the leftover metals in all filtrates.

Lead and copper determination: Flame atomic absorption spectrometer (FAAS) (Polarized Zeeman Atomic Absorption Spectrophotometer ZA3000 Series) was used to examine lead and copper concentrations in contaminated or purified water. Flame atomic absorption spectrometer was furnished with air-C₂H₂ (acetylene) burner which is controlled by a computer. Cathode lamp was adjusted at 7.5 mA and wavelengths for lead and copper were adjusted at 283.3 nm and 324.8 nm, respectively. The reduction in concentrations of lead and copper was interpreted as adsorption percentage which is calculated by using the formula:

$$\text{Adsorption \%} = \frac{(C_i - C_e)}{C_i} \times 100$$

where C_i and C_e are initial and final metal ion concentrations, respectively.

Experimental design and statistical analyses: Response surface methodology (RSM) was used to achieve

maximal information about the process from a minimal number of possible experiments. The type of RSM used in this study was Box-Wilson experimental design to determine the optimal conditions of biosorption process using BPR. The application of a design is a convenient way to optimize a process with three levels (-1, 0 and +1) for each factor (table 1). This design is applied to evaluate the effects and interactions of five independent variables, namely pH (X_1), time (X_2), metal concentration (X_3), bio-sorbent dose (X_4) and temperature (X_5). The selected optimization parameters were the adsorption percentage of lead (Y_1) and copper (Y_2). The experimental design used was constructed and the experimental results were processed by applying ANOVA using the software STATGRAPHICS PLUS (Version 5.1, Statistical Graphics Corporation, Rockville, USA, 2000).

Table 1: Coded and actual levels of independent variables used for optimization of BPR to remove heavy metals from water.

Independent variables	Coded levels		
	-1	0	+1
pH	2	4	6
Time (min)	20	40	60
Metal concentration (ppm)	20	40	60
Bio-sorbent dose (g/L)	1	2	3
Temperature (°C)	20	30	40

Comparative study: The biosorption of heavy metals using BPR at optimum conditions were compared with PBPP using similar levels of all the studied parameters. In addition, the results were also compared with raw banana peel powder to observe the storage effect on total phenolic content and biosorption potential.

RESULTS AND DISCUSSION

Bioactive potential of raw banana peel and preserved banana peel powder: Banana peel is a rich source of polyphenols and several natural antioxidants. Extraction method used in this study was ultrasonic-assisted extraction to get maximum yield of phenolic compounds in short time (Khan *et al.*, 2010). The total phenolic contents of DBPP extract obtained after sonication were 2.69 ± 0.15 g GAE/ 100 g DM. The results of current study correspond well with previous work performed to estimate the phenolic contents of banana peel (Pereira *et al.*, 2017). The comparative study with PBPP showed no significant difference in phenolic contents (2.67 ± 0.11 g GAE/ 100 g DM) which endorses the storage of banana peel powder up to one month at room temperature.

Although little less as reported by other studies (Babbar *et al.*, 2011), the antioxidant activity or FRSA of PBPP was found $76.69 \pm 0.93\%$ similar to DBPP. Antioxidant activity of banana peel might be associated with the presence of polyphenols and flavonoids, as along with carotenoids and vitamins as they also take part in the antioxidant potential. Although methanol extract and other organic solvents gives more yield of phenolic compounds and antioxidants compared to ethanol extract but later is more practical for application as natural preservative in food and beverage industries (González-Montelongo *et al.*, 2010). The remains of this extraction process were used further for biosorption of heavy metals.

Biosorption potential of BPR: A response surface analysis: A response surface statistical strategy was launched to optimize the studied parameters for maximum biosorption of lead and copper using BPR (table 2). Maximum elimination of lead (87.30%) and copper (67.50%) was found for trial 24 and trial 23, respectively, according to the prescribed design. The ANOVA on the obtained data has shown pH and temperature for lead, and with addition of metal ion concentration for copper as significant parameters to affect the biosorption process (table 3). Results obtained from this study shows that lead ions have the highest binding affinity to active sites on BPR surface as compared to copper ions.

Effect of pH: The pH of the solution is most significant factor in metal ion reduction. It influences the surface charges of bio-sorbent and the species of adsorbate that's why it is important controlling parameter in adsorption process. Increase in pH also increases the removal of lead and copper. The interactive effect of pH with time, metal ion concentration, bio-sorbent dose and temperature for removal of lead and copper has shown in estimated response surface plots (figure 1). From response surface methodology, the optimum value of pH at 6 is recommended for maximum removal of lead and copper using BPR. Plant based biomass comprised of different functional groups such as $-OH$, $=C=O$ and $-NH_2$ are responsible for binding lead and copper in aqueous solution (Shi *et al.*, 2016). The maximum abstraction of lead and copper at pH 6 is due to increasing in the amount of negatively charged sites on bio-sorbent surface that enhance the process of adsorption. Different studies showed the effect of solution pH on the biosorption capacity of various biomasses, for instance, groundnut shell for removal of lead at pH 5.6 (Janyasuthiwong *et al.*, 2015), lentil husk for lead removal at pH 5 (Basu *et al.*, 2015) and pomegranate peel for copper removal at pH 5.8 (Ben-Ali *et al.*, 2017).

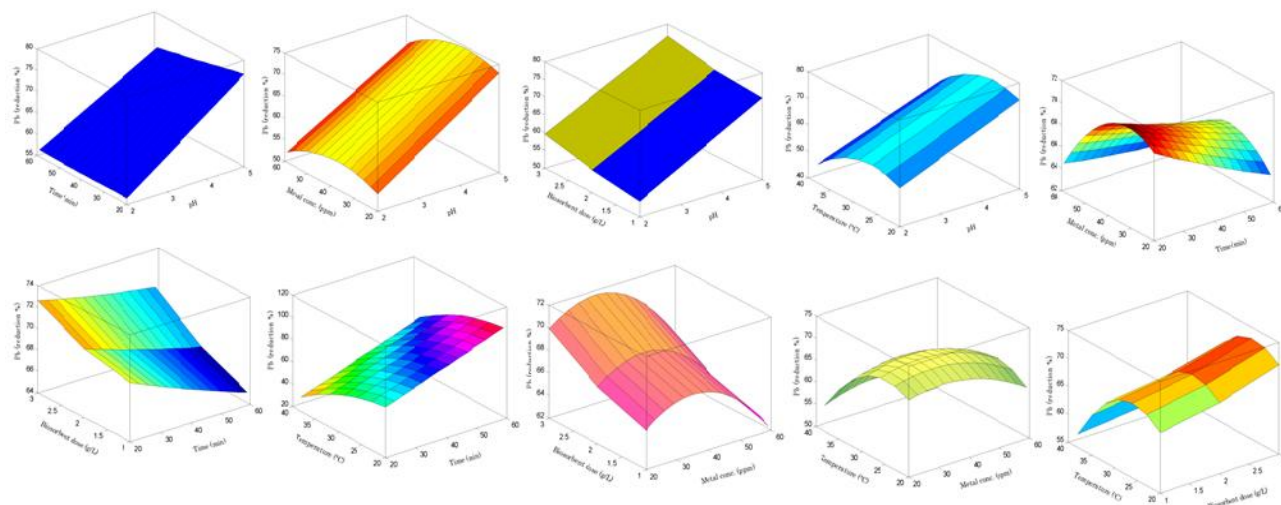


Figure 1. Estimated response surface plots showing the interactive effects of pH with other parameters on the percentage removal of lead and copper using BPR.

Table 2: Percentage reduction of lead and copper from BPR by using different combinations proposed by Box-Wilson design.

Sr. No	Independent Parameters					Response factors			
	pH	Time (min)	Metal conc. (ppm)	Bio-sorbent dose (g/L)	Temperature (°C)	Pb (ppm)	Pb (reduction %)	Cu (ppm)	Cu (reduction %)
1	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.68	68.30	21.44	46.40
2	4 (0)	40 (0)	60 (1)	2 (0)	30 (0)	23.46	60.90	34.80	42.00
3	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.92	67.70	21.80	45.50
4	2 (-1)	60 (1)	60 (1)	3 (1)	20 (-1)	27.48	54.20	41.34	31.10
5	4 (0)	60 (1)	40 (0)	2 (0)	30 (0)	13.56	66.10	20.48	48.80
6	2 (-1)	40 (0)	40 (0)	2 (0)	30 (0)	20.64	48.40	28.88	27.80
7	2 (-1)	20 (-1)	20 (-1)	3 (1)	20 (-1)	8.64	56.80	13.54	32.30
8	2 (-1)	20 (-1)	20 (-1)	1 (-1)	40 (1)	11.70	41.50	16.50	17.50
9	2 (-1)	60 (1)	20 (-1)	3 (1)	40 (1)	11.28	43.60	15.00	25.00
10	6 (1)	20 (-1)	60 (1)	1 (-1)	40 (1)	22.38	62.70	33.30	44.50
11	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.40	69.00	22.04	44.90
12	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.88	67.80	21.32	46.70
13	4 (0)	40 (0)	40 (0)	1 (-1)	30 (0)	14.60	63.50	22.56	43.60
14	6 (1)	60 (1)	60 (1)	1 (-1)	20 (-1)	20.04	66.60	31.50	47.50
15	6 (1)	20 (-1)	60 (1)	3 (1)	20 (-1)	15.48	74.20	26.70	55.50
16	2 (-1)	20 (-1)	60 (1)	3 (1)	40 (1)	34.26	42.90	48.78	18.70
17	4 (0)	40 (0)	20 (-1)	2 (0)	30 (0)	6.10	69.50	9.96	50.20
18	4 (0)	40 (0)	40 (0)	3 (1)	30 (0)	10.00	75.00	18.40	54.00
19	4 (0)	20 (-1)	40 (0)	2 (0)	30 (0)	11.44	71.40	20.40	49.00
20	6 (1)	60 (1)	60 (1)	3 (1)	40 (1)	24.12	59.80	38.70	35.50
21	4 (0)	40 (0)	40 (0)	2 (0)	20 (-1)	9.16	77.10	17.48	56.30
22	2 (-1)	60 (1)	60 (1)	1 (-1)	40 (1)	34.44	42.60	47.90	20.17
23	6 (1)	20 (-1)	20 (-1)	1 (-1)	20 (-1)	3.66	81.70	6.50	67.50
24	6 (1)	40 (0)	40 (0)	2 (0)	30 (0)	5.08	87.30	16.28	59.30
25	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.40	69.00	21.96	45.10
26	6 (1)	60 (1)	20 (-1)	3 (1)	20 (-1)	4.76	76.20	8.30	58.50
27	6 (1)	60 (1)	20 (-1)	1 (-1)	40 (1)	8.50	57.50	11.90	40.50
28	4 (0)	40 (0)	40 (0)	2 (0)	30 (0)	12.08	69.80	21.20	47.00
29	6 (1)	20 (-1)	20 (-1)	3 (1)	40 (1)	6.48	67.60	10.40	48.00
30	2 (-1)	20 (-1)	60 (1)	1 (-1)	20 (-1)	31.86	46.90	41.76	30.40
31	4 (0)	40 (0)	40 (0)	2 (0)	40 (1)	22.00	45.00	30.60	23.50
32	2 (-1)	60 (1)	20 (-1)	1 (-1)	20 (-1)	10.12	49.40	13.62	31.90

Effect of temperature: Temperature is also one of the most significant parameters in adsorption process. With increase in temperature, above 30 °C, adsorption capacity decreases, and increases with decrease in temperature. At temperature of 40 °C, the adsorption of lead reduces to 67.60% and that of copper to 48.00% (table 2, trial 29). But at minimum temperature, 20 °C, rate of adsorption increases for lead and copper up to 81.70% and 67.50%, respectively (table 2, trial 23). The response surface analysis has recommended a temperature of 20 °C for efficient removal of lead and copper using BPR. Siwi *et al.* (2018) reported that temperature above 26 °C causes a decrease in removal of heavy metals due to weak attractive forces between metal ion and bio-sorbent and at temperature 35 °C, the nature of biomass changes, due to these changes active site on the surface of biomass destroyed resulting reduction in biosorption ability.

Effect of metal ion concentration: Metal ion concentration affects the equilibrium of sorption system.

The adsorption percentage of lead and copper onto banana peel biomass was increased with increasing the initial metal ion concentration above 20 ppm. The reason of such trend can be the high bio-sorbent surface area as compared to metal ion concentration in solution. Due to the higher surface area of bio-sorbent more number of active sites are available that can be accessible by metal ions. Interaction of these metal ions with binding sites of sorbent increases the metal removal till equilibrium (Babu and Gupta, 2008). Results obtained from this study shows that metal ion concentration is non-significant parameter for lead adsorption and significant for copper removal. The design suggested an optimum initial metal ion concentration of 20 ppm for lead and copper to be effectively removed using BPR. In fact, a further increase in initial metal ion concentration tends to decrease in adsorption percentage due to inaccessibility of active sites with less bio-sorbent dose (Kumar, 2014).

Table 3: ANOVAs of the predicted second-order polynomial model for lead and copper removal from water using BPR.

Source of Variation	df	Lead (Pb)		Copper (Cu)		
		F-ratio	P-value	F-ratio	P-value	
Linear	A: pH	1	67.50*	< 0.0001	115.37*	< 0.0001
	B: Time	1	1.39 ^{ns}	0.2640	1.39 ^{ns}	0.2625
	C: Metal concentration	1	1.71 ^{ns}	0.2176	4.96*	0.0478
	D: Bio-sorbent dose	1	2.26 ^{ns}	0.1612	0.53 ^{ns}	0.4830
	E: Temperature	1	22.58*	0.0006	44.36*	< 0.0001
Interaction	AB	1	1.37	0.2673	4.83	0.0503
	AC	1	0.40	0.5412	1.67	0.2222
	AD	1	0.11	0.7492	0.24	0.6318
	AE	1	0.37	0.5573	0.69	0.4233
	BC	1	0.53	0.4798	0.08	0.7867
	BD	1	0.14	0.7124	0.63	0.4459
	BE	1	0.01	0.9345	0.22	0.6459
	CD	1	0.01	0.9410	0.18	0.6819
	CE	1	0.71	0.4184	0.49	0.4995
	DE	1	0.09	0.7678	0.05	0.8254
Quadratic	A ²	1	0.04	0.8496	1.39	0.2631
	B ²	1	0.00	0.9663	0.30	0.5973
	C ²	1	0.80	0.3909	0.13	0.7271
	D ²	1	0.03	0.8642	0.26	0.6190
	E ²	1	3.95	0.0723	5.55*	0.0381
Lack of Fit	11	-	-	-	-	
Cor. Total	31	-	-	-	-	
R²	-	-	91.8%	-	94.8%	
R² adjusted	-	-	76.8%	-	85.4%	

*Significant at 0.05 level

^{ns}Non-Significant

Effect of contact time: Experiments performed to determine the equilibrium stage of contact time have recommended first 20 min for maximum removal of lead and copper heavy metals. In fact, with increase in time by keeping other conditions at central point, no significant increment in removal of heavy metals was found. The

results correspond well with previous studies which recommend the binding of heavy metals at bio-sorbent active sites is achieved in 10 min (Castro *et al.*, 2011).

Effect of bio-sorbent dose: Bio-sorbent dose is a non-significant parameter in the process of biosorption of

heavy metals from aqueous solution. Adsorption of heavy metals ion increases with increase of bio-sorbent dose due the availability of more active sites thereby ensuring efficient removal. It was observed from the results of present study that bio-sorbent dose was less affecting factor for lead and copper reduction by biosorption process. The optimum bio-sorbent dose suggested by

design for maximum adsorption of lead was 3 g and for copper was 1g. Further increment in bio-sorbent dose didn't affect the metal reduction and showed respective behavior depending on the metal ion- and this is actually due to equilibrium in metal binding sites and bio-sorbent active sites.

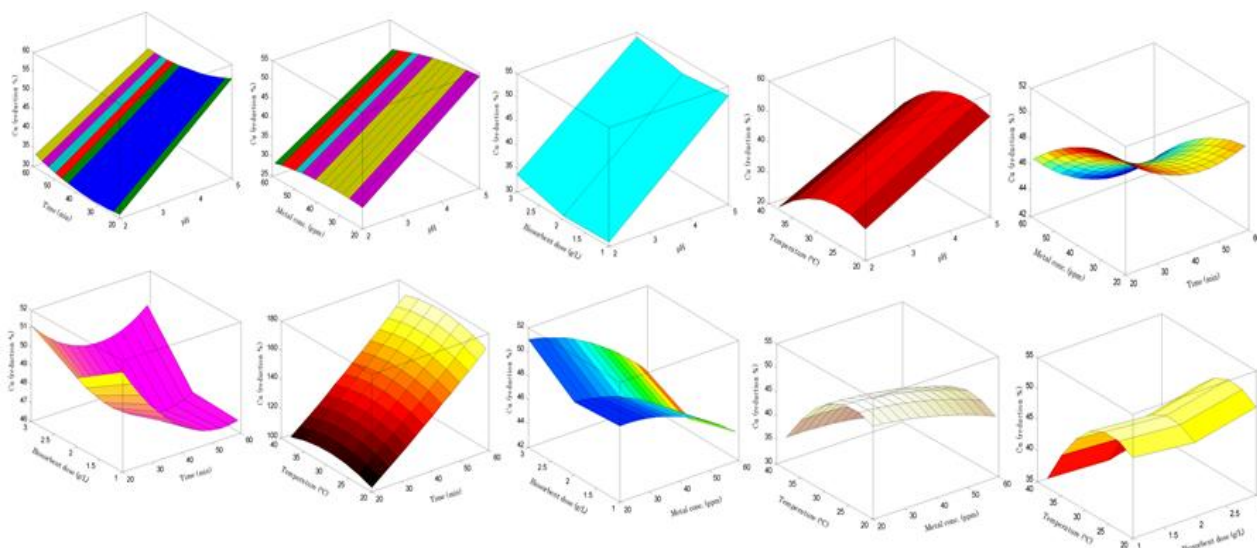


Figure 2. Estimated response surface plots showing the interactive effects of temperature with other parameters on the percentage removal of lead and copper using BPR.

Biosorption comparison between DBPP, PBPP and BPR:

A biosorption comparison has been made between DBPP, PBPP and BPR at optimized conditions. All raw materials were set for biosorption of lead and copper at pH 6, temperature 20 °C, metal ion concentration of 20 ppm, bio-sorbent dose of 3 g/L (Pb) and 1 g/L (Cu), and contact time of 20 min. At optimized conditions, the adsorption capacity of BPR was 87.30% for lead and 67.50% for copper which also validate our predicted optimal conditions obtained from response surface analysis. Compared to BPR, DBPP & PBPP have shown high biosorption percentage of 94.20% & 94.70% for lead, respectively and 73.00% & 72.80% for copper, respectively. Actually, along with other metal binding functional groups such as carbonyl, alcoholic, acetamido, sulphhydryl, amido and amino groups, the phenolic compounds are also good chelating agents to form complexes with the metal ions (Renu *et al.*, 2017). The reduction in lead and copper removal from BPR was due to extraction of phenolic compounds. As results showed that extraction of polyphenols from banana peel had little effect on adsorption capacity of banana peel powder, it can be used even after room temperature storage of one month for both purposes to add value in the discarded waste.

Conclusions: The low cost, locally and easily available banana peel biomass can be used as source of polyphenols as well as bio-sorbent for the removal of heavy metals from water otherwise this waste is accumulating to organic piles. After extraction of polyphenols, the retentate obtained from peel was employed to remove heavy metals from water using response surface methodology. Among all the studied parameters, pH and temperature were most significant to affect the biosorption process. The extraction of phenolic compounds from banana peel slightly affects the potential of retentate for heavy metal removal while storage of preserved banana peel presents no effect on its potential. Therefore, this study suggests the dual usage of preserved banana peel as source of polyphenols as well as bio-sorbent for the removal of heavy metals.

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