INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

MANGOLEAFUTILIZEDAS LOW-COST ADSORBENT FOR THE REMOVAL OF POTASSIUM ION FROM AQUEOUS SOLUTION

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ABSTRACT
The potential of mango leaves (*Mangiferaindica*), as a low-cost adsorbent for the removal of K^+ ions from aqueous solution was investigated in this study. The influences of pH, contact time, initial metal concentration, agitation rate, particle size and effect of biosorbent dosage were studied in batch experiments at room temperature. Maximum sorption for metal was found to be at pH 6.5. Process parameters which include initial metal ion concentration, adsorbent dosages, initial pH and temperature of solution were varied in order to evaluate their influence on the adsorption process. The results obtained indicate that the adsorption of K^+ on MLP is better at higher metal ion concentrations and lower adsorbent dosages. Langmuir and Freundlich isotherm models were fitted to the biosorption of K^+ ions on the mango leaves, it giving correlation coefficient of 0.9991. Langmuir model fitted the equilibrium data better, giving correlation coefficient of 0.9999 and a maximum adsorption capacity of 42.5mg/g. This indicates monolayer coverage on adsorbent. The results showed that mango leaves have the potential to be applied as alternative low-cost biosorbent in the remedy of metal contamination in waste water.

Keywords: Mango leaf powder, K^+ ions, biosorption, adsorption, waste water.

INTRODUCTION

Inmovingtowardsthenewera ofscienceandtechnology,theworldhasseenmajortransformationsinnumerousaspectsoflife.Attheheartofthis,tremendousadvancementliestherapidindustrializationoccurringatvariouspartsoftheglobewhichcausesdetrimentaleffectsformankindandanimals.Onewidespreadphenomenonwhichhasdrawnmuchattentionisthecontaminationoftoxicmetalssuchascopper,lead,zinc,nickel,potassium&chromiumintheaquaticenvironment,whicharesourcedfromchemicalindustriessuchaspetrochemicals,refineries,fertilizers,pulpandpaper[1-3].Heavymetalcontaminationmaycauseserioushealthproblemssuchascancerandbraindamage,duetotheaccumulationinlivingtissuesandorgans[2-4].Generally,metalscouldbedividedintofourdistinguishedcategorieswhicharetoxicmetals,strategicmetals,preciousmetalsandradionuclides.Amongthese,toxicmetalsaretheonesassociatedforcausingseriousenvironmentalthreats,makingitsremovalfromtheaquaticenvironmentessential.Fromthevarioustypesoftoxicmetalspresentinwastewater,Potasssiumwaschosenforthisbiosorptionstudieswithregardtoitswideuseinindustryandpotentialpollutionimpact.Inindustrialwaste,PotassiummainlyappearstobeintheformofthemonovalentK(I),whichismoretoxicthanthemetalitself.Thisisbecauseitissolubleinwaterinitsionicformandcaneasilyabsorbintolivingorganisms.HighdosesofPotassiumintheaquaticenvironmentgeneratetoxologicalconcernsasitcandepositintothebrain,liver,pancreasandmyocardium[3-4].Thus,K^+concentrationsofwastewatershouldbereducedtoavalueofatleast0.2to8mg/LasperWHOguidelinesfordrinkingwater.Theincreaseofmetalbearingeffluentsintotheaquaticenvironmenthascausedprogressivedevelopmentsinwastewatertreatment.Atypicalwastewatertreatmentplantisdividedintos

everalareasandtheremovalofmetallicpollutantsis performedmainlyinthetertiarystage.Currentdevelopedmethodsinthisstageincludefiltration,ionexchange,membraneseparation,nutrientstrippingandadsorption[1,7].However,thesemethodshavesomelimitationsduetotechnicalandeconomicalconstraints.Theionexchangeprocessneedsahighoperationalcostbecauseitinvolvestheuseofexcessiveamountsofreagentforitsresinregeneration.Italsorequiresa highcapitalcostfortheequipmentused.Meanwhile, themembraneprocessapplicationislimitedbytheconditionofthemembraneused.Thismembranetends tobeunstableinsaltyoracidicconditionsandfoulsby organicorinorganicsubstancepresentinthewaste water.Themajordisadvantagefortheprecipitationprocessisthatitgeneratesatoxicsludgethatrequires carefuldisposalasperregulations.Forelectrolysis,itsinefficiencyatlowconcentrationswhichcausesincompletemetalionrecoverywhichisoneofitsdisadvantages[8].Duetothesefactors,researchhasbeenconductedtostudytheuseofnaturaladsorbentsasanalternative,basedontheeconomicalandenvironmentalpointofview.Themostcurrenttechniqueemployedformetalremovalfromwastewaterisbiologicaladsorptionorbiosorption.“Biosorption”isthetermgiventothepassivesorptionand/orcomplexationofmetalionsbybiomass[9].Itisareversibleprocesswhereconcentrationofmetalionsinsolutiondecreasesduetoadsorptionontothesolidphaseuntiladynamicequilibriumbetweentheaqueousandsolidphaseisestablished.Thistechnologywhichutilisesnaturalbiomassmaterialsisveryeffectiveforthedetoxificationofmetal-bearingindustrialeffluents.Thebiosorptionprocess involvesseveralmechanismsthatdifferqualitatively andquantitatively,dependingontheoriginofthebiomass,thespeciesusedanditsprocessing[10].These mechanismsaregenerallybasedonphysico-chemicalinteractionsbetweenmetalionsandfunctionalgroupspresentonthecellsurface,whichinclude ionexchange,complexation,electrostaticattraction andmicroprecipitation[11].Theprocessofbiosorption hasmanyattractivefeaturesincludingtheselectiveremovalofmetalsoverabroadrangeofpHandtemperature,itsrapidkineticsofadsorptionanddesorptionandlowcapitalandoperationcost.Duetohigheraffinityofthesorbentforthesorbatespecies,thelaterisattractedandremovedbydifferentmechanisms.Theprocesscontinuestillequilibriumisestablishedbetweenamountofsolid-boundsorbatespeciesanditsportionremaininginsolution.Varioustypesofbiomasshavebeenusedasthebiosorbentfortheremovaloftoxicmetals.Amongthese,plantleavesarechoseninthisstudyastheyareproposedtobenatural,simpleandcheapbiosorbents fortheefficientremovalofseveralheavymetalions.Theycanbeeasilyfoundandarearenewablesource. Plantleavesarealsonontoxicandbiodegradable.Afterthebiosorptionprocess,theyareexpectedtoprecipitateandbecomesedimentswhichcanbedisposedsafely.Mulchplantleaveswereusedforthebiosorptionofcadmium,leadandcopperions.Theresultsprovedthattheperformanceoftheleavesisclosetotheefficiencyofusingactivatedcarbon.Theresultsalso showedthatovendriedleaveshavebetterperformancecomparedtothenaturallydriedones.Thefunctionalgroupwhichiscommonlyfoundinplantleavesiscarboxylate[9].Availableliteratureinvolvingtheuseof mangoleafpowder(MLP)forthebiosorptionofheavymetalsisquitelimited.Particularly,nostudyinvolvingMLPfortheremovalofPotassiumionwasfound.Onepaperwasfoundinwhichtheresearcherusedseveraltestplantmaterialsincludingplantmaterials includingMLPasthebiosorbentsfortheremovalofleadions[12-18].

AIM AND OBJECTIVES

1. Tofindaninexpensiveandeffectiveadsorbenttoreplacecommercialmethodsinremovingheavymetalsfromaqueouseffluent.
2. ToinvestigatethepossibleuseofmangoleavesasalowcostadsorbentforremovalofK^+ions.
3. TostudytheeffectofpH,effectofinitialconcentrationofK^+ions,contacttime,effectofagitationrate,effectofparticlesizeandeffectof chelatingagentontheadsorptionofK^+ionsonmangoleaves.

MATERIAL AND METHODS

Preparation of biosorbent

Matureandfreshmangoleavesas figure 1werecollectedfromlocaltreesandwashedthoroughlybyusingdistilledwatertocleanthemfromdirtandimpurities.Afterthat,theleavesweresundriedforadayonaperforatedtrayuntiltheleavesturnedbrownishincolour.Theleaveswerethendriedfurtherinanoven (Labtech,EIE-101)for24hoursuntiltheleavesbecamecrisp.Afterdrying,theleavesweregroundbyamechanicalgrinder(Premir,Xprees750),toconstantsizeof80μmandtheresultingMLPwaskeptinaglassbottlereadyforfurtherexperiments.

Preparationofadsorbate

Thepotassiumstocksolution(1000mg/L)wasprepared**,**whichwaspurchasedfromSuvidhinath laboratories.Necessarydilutionsweredonetoobtain50mg/L,75mg/Land100mg/Lofpotassiumionsolution.Standardconcentrationsof10,20,30mg/LofK^+solutionswerealsopreparedforcalibrationpurposes.Theinitialandfinalmetalconcentrationswerean alyzedviaFlamePhotometry(Chemiline,Cat#:CL410)usingtheflameatspecificcondition.ThepHofthe K^+solutionwasadjustedbyadding0.1MHClor0.1MNaOHandthemeasurementwasdonebypHmeter(Chemline,ATCCL120).

Biosorptionexperiments

ThebiosorptionofK^+fromaqueoussolutionwasinvestigatedinbatchbiosorptionexperiments.TheMLPwithdifferentdosages(0.4,0.6,0.8,1.0and1.2g/L)wereaddedeachin5conicalflaskscontaining100mLof100mg/LKClsolution.5mLoftheKClsolutionwaswithdrawnandaddedinvolumetricflaskthatcontained45mLofdeionisedwaterforthepurposeofdilution. Afterthat,thebatchshakeflaskexperimentswereperformedusingasonicator(Toshconultrasoniccleaner,ToshniwalinstrumentPvt.Ltd.Ajmer)with75rpmat30°Cfor3hours.Forthefirsthour,thesampleswerewithdrawnevery15minutes.Forthefollowinghours,thesameprocedurewasrepeatedatevery30minutesinterval.ThesamplesweretestedfortheirK^+ionconcentrationwiththeFlamePhotometry. ForpHexperiments,thepHofthesolutionwasadjustedto2.5,4.5,8.5and10.5byusing0.1MHCland0.1MNaOH.FivedifferentsetsofexperimentswithvariableconcentrationofK^+solution,pHandtemperaturewereperformed. Theamountofmaterialadsorbedperunitmassofadsorbent(mg/g)isgiveninequation(1):

$$q = \frac{Co - Ct}{m}\text{.......................(1)}$$

Where,Coistheinitialconcentrationofadsorbate(mg/L)andCtistheconcentrationofadsorbateattime(mg/L). Theadsorptionefficiencycanbeexpressedaspercentageadsorptionofmetalionperequation(2):

$$\%adsorption = \frac{Co - Ct}{Co} X100\%\text{..................(2)}$$

Where,CoandCtaretheinitialadsorbateconcentration(mg/L)andtheadsorbateconcentrationattime,t(min)respectively.

Equilibriumstudies

Equilibriumdata,commonlyknownasadsorptionisotherms,arebasicrequirementsforthedesignofadsorptionsystems.Classicaladsorptionmodels,Langmuir(1918)andFreundlich(1907),wereusedtodescribetheequilibriumbetweenpotassiumionsontheMLPatconstanttemperature. TheLangmuirequationisvalidforamonolayersorptiononahomogenoussurfacewithafinitenumberofidenticalsitesandwhentherearenointeractionsbetweenthesorbedspecies.ThelinearformofLangmuireequationisgiveninequation(3):

$$\frac{Ce}{qe} = \frac{1}{qm} * Ce + \frac{1}{Kl}(qm)\text{.................................(3)}$$

whereCe(mg/L)istheequilibriumconcentrationofadsorbate,qe(mg/g)isthequantityofadsorbedmaterial(mg/g)atequilibrium,KListheLangmuirequilibriumconstantrelatedtotheenergyofsorption(Lmg^{-1})andqmisthemaximumamountofmetalionsperunitweightofMLPtoformacompletemonolayeronthesurfaceboundathighCe.Italsorepresentsapracticallimitingadsorptioncapacitywhenthesurfaceisfullycoveredwiththemetalionsandassistsinthecomparisonofadsorptionperformance,particularlyincaseswherethesorbentdidnotreachitsfullsaturationinexperiments[16-18].

TheempiricalFreundlichequationappliestomultilayersorptiononaheterogeneoussurfaceandcanonlybeemployedinthelowintermediateconcentrationranges.TheFreundlichequationisgiveninequation(4):

$$\log qe = \frac{1}{n} logCe + logKf\text{.............(4)}$$

wheretheKf(mgg^{-1})andn(valuebetween0and1)aretheFreundlichconstantcharacteristiconthesystem.Kfandnareindicatorsforadsorptioncapacityandadsorptionintensity,respectively[17,19-26].

RESULTSANDDISCUSSION

1)EffectofpH:

ThepHoftheadsorbatesolutionisconsideredoneofthemostimportantfactorsaffectingthebiosorptionprocess.Thisfactoriscapableofinfluencingnotonlythebindingsitedissociationstate,butalsothesolutionchemistryofthetargetmetalintermsofhydrolysis,complexationbyorganicand/orinorganicligandsandredoxpotentials. TherangeofpHtestedinthisexperimentwasfrom2.5to10.5.SolutionswithpHlowerthan2.5wasalsonotsuitableasforthesesolutions,thesurfaceactivesitesoftheadsorbentwouldbeprotonated,resultinginacompetitionofk^+andH^+ionsforthesamesurfaceactivesiteswhichwouldresultinalowpotassiumuptake[4,27-29].

Inthepresentstudy,thepHwasadjustedintherangeof2.5-6.5byusingdiluteH_2SO_4and8-10.5byusingNaOH.Theexperimentswerecarriedoutat50mg/L,75mg/L,100mg/Lofinitialmetalionconcentrationwith1.2g/LMLPdosage.DrawthegraphofpHvs%adsorptionaccordingtotable1.FromFig.2,itcanbeobservedthattheadsorptionishighestforpH

6.5followedbypH.TheseresultswerealsopresentedinmanypreviousstudieswhichinvolvedamaximumadsorptionfrompH4.5to5.5.ThistrendwasexpectedasatlowpHvalues,mostofthebindingsitesontherootsurfacewouldhavepositivechargesmakingthebindingandsorptionofthemetalionsunattractivetothosesites[30-32].

ThecompetitionofK^+withH^+ionsforappropriatesitesontheadsorbentsurfacelowerstheadsorptionofK^+ions.However,asthepHofthesolutionincreases,thiscompetitionweakens,whereK^+ionsreplaceH^+ionsboundtotheadsorbentsinceincreasingnumberofprotonsaredissociatedfromfunctionalgroupsonthecellwall.Inaddition,athigherpH,therewillbeachangeinthedominantfunctionalgroupsresponsibleforbindingonthecellwall,withcarboxylategroupsanddiaminegroupsbeingthemostimportantonesfor$pH<5$andpH6respectively[35].MorenegativegroupswerethenmadeavailableontheadsorbentsurfaceaspHincreases.

Thenegativelychargedadsorbentsurfaceincreasedtheelectrostaticattractionbetweenpositivelychargedadsorbateandnegativelychargedadsorbentparticlesandtherefore,leadingtoanincreaseintheadsorptionofK^+ions.However,furtherincreaseinthepHwillcausethemetalstoformprecipitates.

2)EffectofTime:

TheeffectofcontacttimewasstudiedatdifferentinitialmetalionconcentrationsandMLPdosages.Itcanbeclearlyobservedthatthepercentageofadsorptiongenerallyincreaseduntiltimereached120minutes.Afterthistime,therewasadropintheadsorptionpercentage.Thus,120minuteswaschosenastheoptimumtimewheretheadsorptionreachedequilibrium.

Thehighestpercentageofadsorptionwasfoundtobe31.86%,27.36%and54.54%forconcentrationof50mg/L,75mg/Land100mg/Lrespectively.Fromtheconcentrationexperiments,100mg/LofK^+wasfoundtobeoptimalforthedosageofMLPused.TherelationshipofadsorptionefficiencywithtimeatvariedMLPdosagesisdepictedinFig.3 according to table 2.Theresultsrevealasimilartrend,whereMLPremovalishigheratthebeginning(forthefirst45minutes)forallthedosagesused.

ThisisduetothelargersurfaceareaofMLPavailableduringtheinitialcontacttime.Asthesurfaceadsorptionsitesbecomeexhausted,theuptakerateiscontrolledbytherateatwhichtheadsorbateistransportedfromtheexteriortotheinteriorsitesoftheadsorbentparticlesSimilarresultswerereportedusingwheatshellasthebiosorbent.FiguresshowthattheinitialmetalionconcentrationandMLPdosagedidnotaffectthetimefortheadsorptiontoreachitsoptimumtimeof120minutes.Aftertheoptimumtime,bothplotsalsoshowedadecreaseintheremovalefficiency.Thisindicatesthatdesorptionprocessmayhaveoccurred,asadsorptionisareversibleprocess[36-45].

3)Effect of Metal Ion Concentration

Theinitialmetalionconcentrationplaysanimportantroletowardstheperformanceofabatchbiosorption.TheeffectcanbestudiedfromtheresultsofexperimentsatconstantdosagesofMLP.As shown in Fig.4,themetaluptakeofdifferentdosagesofMLPincreasedastheinitialmetalionconcentrationincreasedfrom50to100g/L,wherethemaximumvalueofmetaluptakewasobservedtobefrom42.5mg/gto7.5mg/grespectively.Thesevalueswereobtainedattheoptimumcontacttimeof120minutesandMLPdosageof0.4g/L.Thevalueof42.5mg/gisthemaximummetaluptake,orthemaximumadsorptioncapacitythatwasachievedbythebiosorptionofK^+onMLPinthisstudy.Thisobservationwasalsoinlinewiththeresearchperformedbytwootherresearcher[46-48].

Theresultisexpectedastheinitialmetalionconcentrationfunctionsasthedrivingforcetoovercomemasstransferresistancesbetweentheaqueousandsolidphases.Inaddition,theincreaseintheinitialmetalionconcentrationalsoincreasedthenumberofcollisionsbetweenthemetalionandthebiosorbent,henceincreasingthemetaluptake.Thus,itcouldbeconcludedthatthehighestmetaluptakewouldoccuratthehighestmetalconcentration,whichis100mg/L,atoptimalconditions[49].

4)EffectofBiosorbentDosage

Theinfluenceofthebiosorbentdosageontheprocesswasexaminedbyusingfivedifferentdosagesat0.4,0.6,0.8,1.0and1.2g/L.Fig.5showstherelationshipofmetaluptakewiththebiosorbentdosageatK^+ionconcentrationof100mg/Landtimeof120minutesaccordingtotable4.Itcanbeobservedthattheincreaseinthebiosorbentdosagecausesdecreaseinthemetaluptake.SimilarresultswerealsoobservedforK^+concentrationsof50mg/Land75mg/L.Thisphenomenonisduetothesplittingeffectofflux(concentrationgradient)betweentheadsorbateandbiosorbentwithincreasingbiomassconcentrationcausingadecreaseinamountofmetallicionadsorbedpergramofbiomass.

Anotherfactoristhatathighsorbentdosages,theavailablemetalionsareinsufficienttocoveralltheexchangeablesitesonthebiosorbent,usuallyresultinginalowmetaluptake[50].Besides,itmightduetotheformationofaggregationduringbiosorptioncausingadecreaseintheeffectiveadsorptionareawhenthebiomassconcentrationincreases.Thus,itcouldbeconcludedthatforthisstudies,thehighestmetaluptakeoccursatthelowestbiosorbentdosage(0.4g/L)ifotherprocessconditionsarekeptconstant[51].

5)Adsorptionisotherms

Manymodelshavebeenproposedtoexplainadsorptionequilibria,butthemostimportantfactoristohaveapplicabilityovertheentirerangeofprocessconditions.Themostwidelyusedisothermsforsolid-liquidadsorptionaretheLangmuirandFreundlichisotherms.Bothoftheseisothermsrelatetheadsorptiondensity,qe(metaluptakeperunitweightofadsorbent)toequilibriumadsorbateconcentrationinthebulkfluidphase.Langmuirisothermmodelisvalidformonolayeradsorptionontoasurfacecontainingafinitenumberofidenticalsites.Thisisothermisderivedfromtheassumptionthatamaximumadsorptioncorrespondstoasaturatedmonolayerofsolutemoleculesontheadsorbentsurface,withconstantenergyofadsorption,andnotransmigrationofadsorbateintheplaneofthesurface[52-53].ThecorrelationcoefficientthatwasobtainedfromtheLangmuirplotasshowninFig.6is0.999.ThistrendisalsoobservedforadsorptionatdifferentMLPdosagesandinitialmetalionconcentration.ThesevaluesprovethattheadsorptionisfavorableforaLangmuirisotherm.Freundlichisothermgivestherelationshipbetweentheequilibriumliquidandsolidphasecapacitybasedonmultilayeradsorption(heterogeneoussurface).TheFreundlichisothermisbasedontheassumptionthattheadsorptionsitesaredistributedexponentiallywithrespecttotheheat.BasedonFig.7,thecorrelationcoefficientthatwasfoundfromtheFreundlichplotis0.9991.ThishighvalueindicatesthattheadsorptionisfavorableforaFreundlichisotherm.Inaddition,theadsorptionintensity,n,whichwasfoundtobe0.625,furtherprovesthisfinding.Thisisasthisvalueissmallerthan1.Meanwhile,theFreundlichconstant,KFwasfoundtobe1.628.TheseresultsalsoindicatethattheMLPsurfaceisheterogenousinthelongrange,butmayhaveshortrangeofuniformity[54].

CONCLUSION

ThefollowingconclusionscanbedrawnbasedontheinvestigationofK$^+$adsorptionbyMLP:

1. ThemaximumadsorptioncapacityofK$^+$onMLPwas206.85mg/gwhichwasobtainedwith0.4g/LMLPdosage,100mg/LK$^+$concentrationandapHof4.5.
2. TheoptimumcontacttimeforthemaximumadsorptioncapacityofK$^+$onMLPwas120minutes.
3. ThemetaluptakeofK$^+$onMLPdecreasedwithincreasingMLPdosage.
4. ThemetaluptakeandadsorptionefficiencyofK$^+$onMLPincreasedwithincreasingmetalionconcentration.
1. 5.TheexperimentaldatafortheadsorptionofK$^+$onMLPfitswellfortheFreundlichisotherm.


(a) (b)

Fig. 1: (a) Natural mango leaves (b) Dried mango leaves

Table 1: Effect of pH in K^+ ion concentration

Sr.no	pH	Conc. Of k+ ion (mg/ml)		
		50	75	100
1	2.5	16.48	13.68	19.19
2	4.5	25.27	23.15	38.38
3	6.5	32.96	29.47	52.52
4	8.5	29.67	27.36	45.45
5	10.5	15.38	17.89	36.36

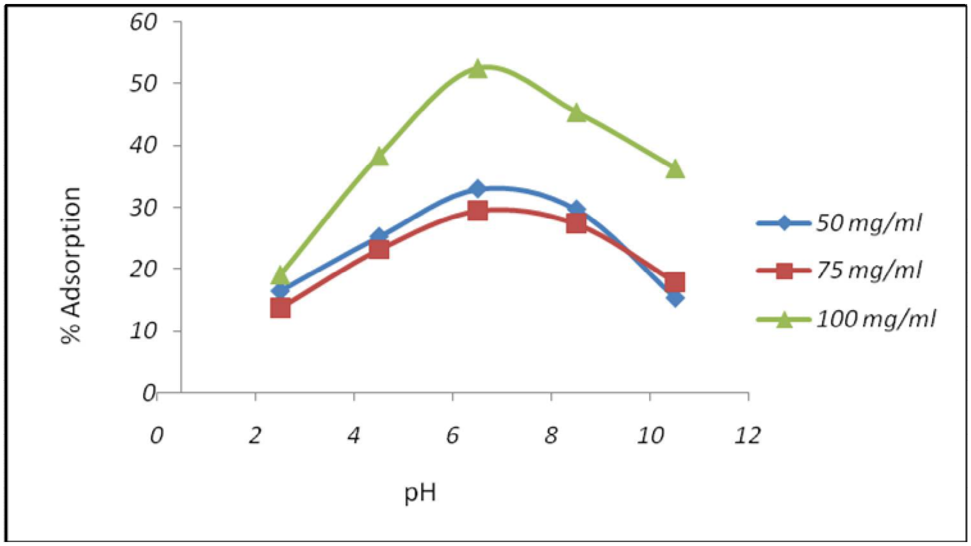

Fig. 2: Plots of adsorption of K^+ ion(50, 75, 100 mg/L) on MLP (1.2 g/L)using various pH

Table 2: Effect of time in K^+ ion concentration

S.No.	Time (min)	Conc. of K^+ ion (mg/ml)		
		50	75	100
1	0	91	95	99
2	60	12.08	9.47	8.08
3	120	31.86	27.36	54.54
4	180	24.17	20	17.17

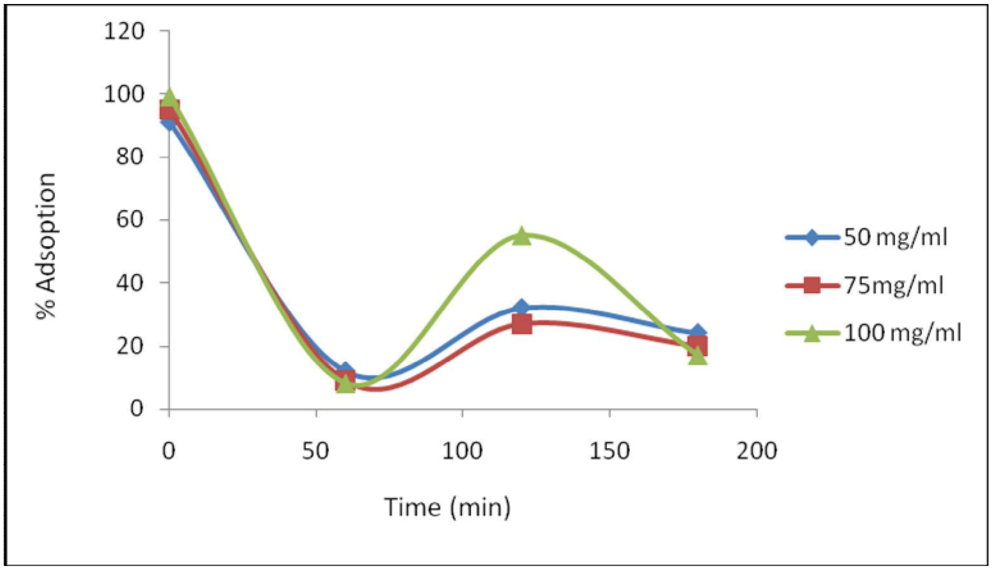

Fig. 3: Plots of adsorption of K$^+$ ion(50, 75, 100 mg/L) on MLP (1.2 g/L)using various time interval (60, 120 and 180 mins)

Table 3: Effect of concentration in metal ion uptake

Concentration	Metal uptake				
	I	II	III	IV	V
50	5.1	6	8.12	19.2	37.9
75	6	4	10.6	20.21	40.6
100	7.5	7	12.5	23.33	42.5

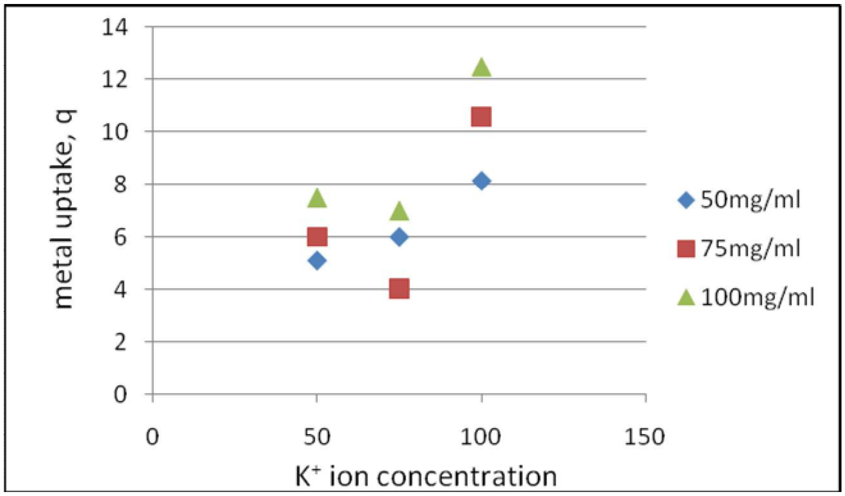

Fig.4: Plots of metal uptake, q against K$^+$ ion concentration withdifferent MLP dosages at 30^{o}C

Table 4: Effect of biosorbentdosage form in metal ion uptake

S.No.	Con. Of MLP (mg/ml)	Metal Uptake(q)in %
1	0.4	42.5
2	0.6	23.23
3	0.8	12.5
4	1.0	7
5	1.2	7.5

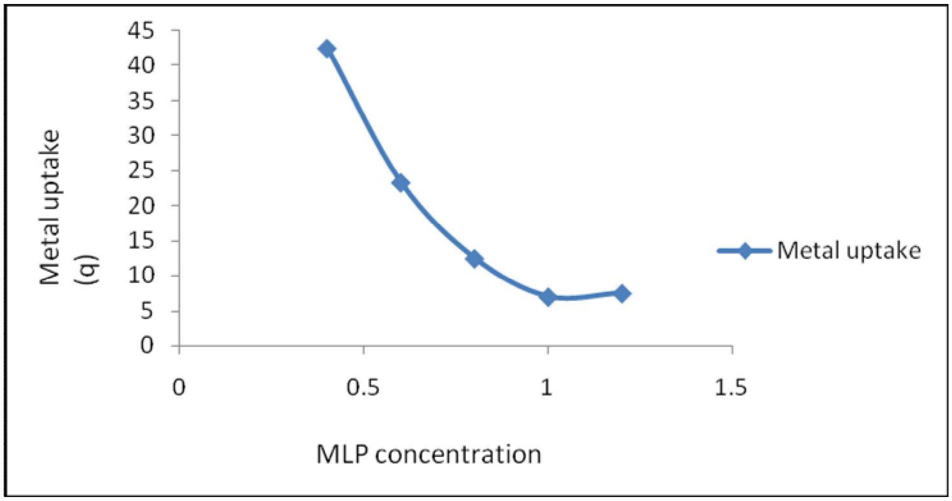

Fig.5: Plot of metal uptake, q against MLP dosage at 100 mg/L K^+ concentration at 30°C

Table 5: Langmuir adsorption isotherm

Ce/Cq (mg/ml)	Ce (mg/ml)
1.36	42.5
0.91	23.33
0.65	12.5
0.52	7
0.54	7.5

Table 6: Freundlich adsorption isotherms

log qe	log Ce
2.64	1.62
2.47	1.36
2.3	1.09
2.16	0.84
2.17	0.87

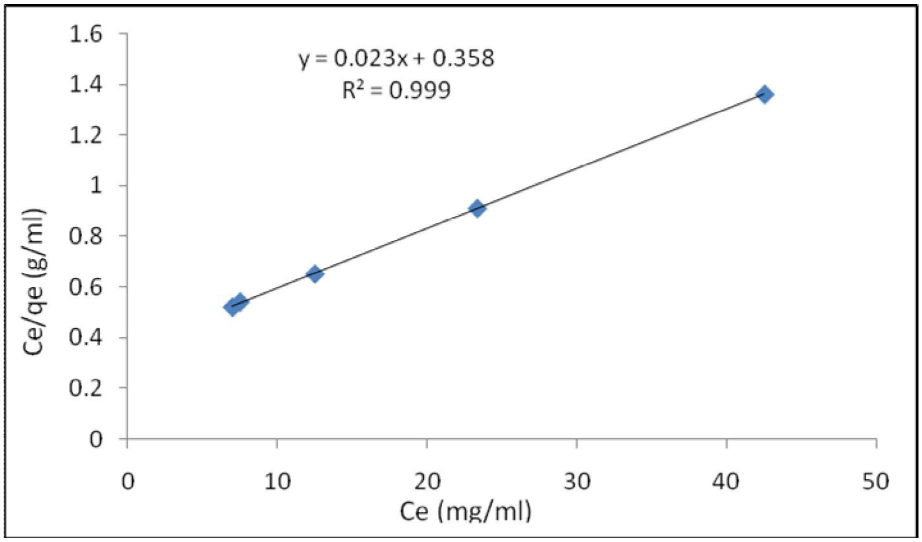

Fig. 6: Langmuir plot for adsorption of K^+ on MLP with dosage of 0.4 g/L and at 30°C

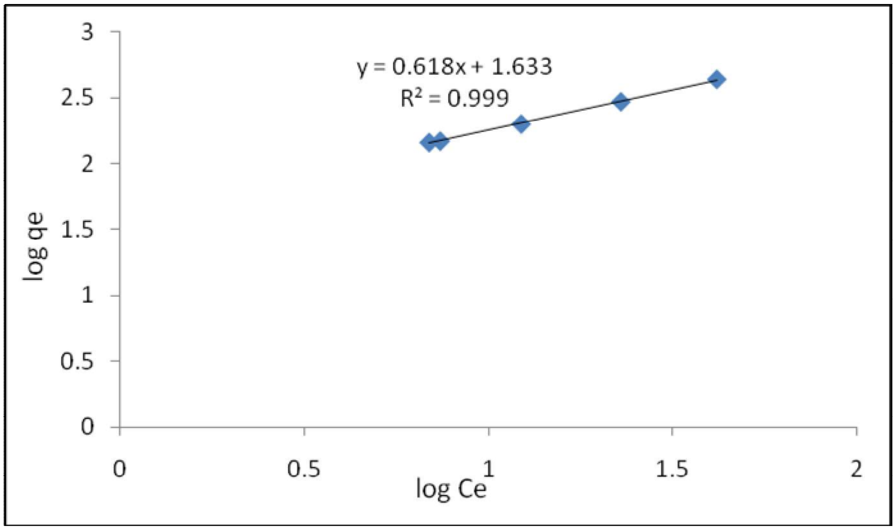

Fig. 7: Freundlich plot for adsorption of K^+ on MLP with dosage 0.4 g/L and at 30°C

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