

Development and Adsorption Study of Macro fungus Biocomposite for Removal of Metal Pollutants



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CERTIFICATE

It is to certify that this dissertation submitted by Ms. Farah Farooq is accepted in its present form by the Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, as satisfying the dissertation partial requirement for the award of degree of PhD in Environmental Sciences.

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ABSTRACT

The identification of toxic metals in Macrofungus species diverted the attention of the researchers for the development of remediation models. Mushrooms represent an important food commodity cultivated in different parts of the world. The present study is designed to exploit the property of metal accumulation in the chitin wall of Mushrooms (*Pleurotus ostreatus*) for the development of biocomposite. A series of novel composites are prepared using calcium alginate, silica and alumina separately and in combination of 10:1 with Macrofungus.

Each composite is characterized through FTIR, SEM and EDX to determine the surface and bulk characteristics. The FTIR analysis revealed –CH, C–O–C (sugar), C=O conjugation and –NH functional groups confirming the proteinaceous nature of Macrofungus. SEM and EDX also confirm the successful preparation showing significant elemental composition of Ca, Si, and Al in respective composites. BET analysis indicated higher surface area for inorganic precursors (SiO₂ and Al₂O₃) than organic moiety (calcium alginate).

The prepared composites are applied as adsorbents in batch mode for the removal of three selected metals (Lead, Cadmium and Chromium) also known as cumulative poisons. For adsorption experiments, optimization of pH is also attempted.

The results are encouraging showing optimum removal of 99% during contact of 45 minutes between the adsorbate and adsorbent. It is also noted that functionalized composites (FMF, FMFSi, FMFAl, FMFSiAl) show relatively less adsorption of metals likely due to stabilizing effect of inorganic precursors (Silica and alumina) and immobilization of Macrofungus by calcium alginate. The metal removal follow the sequence Cr>Cd>Pb in accordance with position of elements in the periodic table.

Kinetics and isotherms were applied for each metal and each adsorbent. Freundlich and Temkin isotherms fitted best to the adsorption data.

The study recommends that the prepared biocomposites can effectively be applied as remediation model for the removal of metals and other pollutants as well.

CHAPTER 1 - INTRODUCTION

Mushrooms are an important class of Fungi with great significance. They are popular food consumed in almost every part of the world. The consumption is reported to exceed 10kg for some individuals (Kalac & Svaboda, 2000). The cultivation and growth of Mushrooms is a source of earning for the farmers and therefore, its cultivation is reported to increase remarkably during the last few decades (Royse, 2002, Sanchez et al., 2002). Mushrooms are regarded as natural recycler that can be grown on variety of waste media under various agro-climatic conditions (Jandaik & Goyal, 1995).

Pleurotus species are most popular and widely cultivated especially in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al.*, 2007). The chemical and nutritive value (Manzi et al., 1999) of mushrooms is clearly understood and is attributed to the chemical constituent. *Pleurotus* species are rich source of proteins, minerals (Ca, P, Fe, K and Na) and vitamin C, B-complex (Çağlarırnak, 2007). *Pleurotus* sp. contains high potassium to sodium ratio, which makes mushrooms an ideal food. Mdachi *et al.*, (2004), showed that species of *Pleurotus* are rich in glutamic acid, aspartic acid, lysine, leucine, valine, arginine and threonine.

On the contrary, the identification of toxic metals (Isiloglu et al., 2001; Kalacetal., 1991; Kalac et al., 1996) in the content of wild edible mushrooms is alarming and can't be ignored. Many mushroom species are known to accumulate high levels of cadmium, mercury and lead (Kalac & Svaboda, 2000). Numerous studies report that both cultivated and wild fruit bodies of mushrooms can accumulate high amounts of heavy metals.

1.1. Pollution Load by Metals

Metals are long known as non-degradable and persistent components of the Earth's crust (Stratton, 1987). Chemical and physical weathering of igneous and metamorphic rocks and soils releases metals into the sediment and into the air (Kennish, 1992). The decomposition of plant and animal detritus, precipitation, deposition of airborne particles from volcanic activity, wind erosion, forest fire, oceanic spray, and surface runoff contains high levels of metals such as iron, manganese, zinc, copper, nickel and cobalt.

These and other contributions add metals in different environmental compartments as Pollution load. As a result, the natural balance of biogeochemical cycles changes considerably. Further, human activities, industrial discharges and waste sludge substantially contribute to metal loading in surface and groundwater supplies. Consequently, concentration of these metals is continuously increasing in environmental media as a matter of great concern all over the world. The increase is due to wide application of these elements in electroplating, leather, jewelry, electric, electronic, metallurgical, and mining industry in industrial nations, and arts and crafts in developing countries (Miroslav et al., 2008). Currently, anthropogenic inputs of metals go beyond the natural inputs.

All metals are not toxic, some of them like cobalt, copper, zinc, and selenium is essential for metabolism of human body in small quantities (Bánfalvi, 2011). The toxicity of metals to the biota (Butter et al., 1996) is associated with mobilization in the food chain.

Chromium in its two stable oxidation states has contrasting toxicity, mobility and bio availability. Whereas Cr (III) is essential in human nutrition (especially in glucose metabolism), Cr (VI) is considered poisonous and has chronic toxicity (Gardea-Torresdey et al., 2000). Dermal exposure to chromium causes irritation, allergic contact dermatitis and hepatic failure in case of acute poisoning. Cr(III) is about 300 times less toxic for the reason that it is poorly absorbed by any route (Yao et al., 2008). The World Health Organization (WHO) has determined that Cr(VI) is a human carcinogen.

Chromium and its compounds are widely used in electroplating, leather tanning, cement, dyeing, metal processing, wood preservatives, paint and pigments, textile, steel fabrication and canning industries. These industries produce large quantities of toxic wastewater effluents (Babel et al., 2004; Faria et al., 2004). The maximum concentration limit for Cr (VI) for discharge into inland surface waters is 0.1 mg/l and in potable water is 0.05 mg/l.

Cadmium a highly toxic metal has an estimated elimination half-life of 10–30 years. It accumulates in the human body mainly in the liver and the kidney (Tim et al., 2008). Chronic exposure to elevated level of cadmium cause high blood pressure, kidney damage, destruction of red blood cells, risk of fractures (Jan et al., 1999), and can disrupt protein metabolism (Vimala & Das, 2011). Cadmium has been added to the list of acknowledged endocrine disrupting chemicals (Chedrese et al., 2006). High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso,

2008) and polluted air (Nordberg et al., 2007). Cadmium is widely present in wastewater from industrial sources such as electro plating, mining, batteries, paint pigments, iron and steel manufacturing (Holan et al, 1993; Volesky et al, 1993; Chong, 2000).

Lead is another highly toxic threat to humans, plants and animals (Low et al., 2000). Its poisoning is manifested through behavioral problems, learning disabilities, seizures and even death. The toxic symptoms are headache, anemia, insomnia, irritability, hallucination, weakness of muscles, and renal damage (Naseem & Tahir, 2001). The lowest reported lethal dose (LD₅₀) in human is 1470 µg/Kg (Sax & Lewis, 2004).

1.2. Metal Pollution Management Methods

Metals pollution in soil, air, estuaries, rivers and oceans locally or globally is the result of untreated waste water. Treatment of wastewater due to stringent environmental awareness is of paramount importance. The commonly employed treatment processes for effluents are precipitation (Mahmood et al., 2011), ion exchange (Xing and Chen, 2007), adsorption (Zimmermann et al., 2010), membrane separation (Das et al., 2006), biological treatment (Park et al., 2006), solid phase extraction (Wang et al., 2011), electrochemical (Martinez et al., 2004), reverse osmosis (Nomanbhay & Palanisamy, 2005), nanofiltration and adsorption on activated carbon (Vinodhini & Das, 2010), ultra filtration (Bernard and André, 1988). Activated carbon and synthetic resins completely remove pollutants but at huge cost. Activated carbon preparation by pyrolysis (400–800°C) followed by activation (800–1000°C) is energy extensive process (Pagnanelli et al., 2008). The sludge in biological treatment results conversion of aquatic problem to solid disposal problem. Inefficiency of precipitation for dilute solutions (Ceribasi & Yetis, 2001), high maintenance cost for ion exchange (Rao et al., 2002), and prolonged Phytoremediation technique (Cheng, 2003) lead to look for new and improved clean technology for reclamation of metal contaminated waters.

Metal pollution is treated with a number of chemical and physical processes. However, the existing processes are not economical viable and need high technological orientation for treating dilute metal concentration.

In this situation adsorption emerged as innovative technology since 1980's (Bakkaloglu et al., 1998). This striking alternative chiefly comprises cell surface complexation, ion exchange and micro precipitation (Gadd, 1990; Muraleedharan et al., 1991). It is attractive because of its near to

the ground price, exceptional ease, high competence, and potential resurgence and reclaim of metals (Zhao et al., 2010; Uguzdogana et al., 2010). Potential incentives for considering biosorption as a viable clean-up technology is its efficiency in dilute effluents and no additional nutrient requirements (Kratochivil and Volesky, 1998). This quest led to discover cost effective adsorbent to offer elevated sorbing efficiency. A variety of materials were tested and implemented for the removal of metals from liquid effluents.

Biomass is a quite simple word for all organic materials that seems from plants, trees, crops and algae. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. Availability is a major factor to be taken into account to select biomass for clean-up purposes. Based on economy and abundance biomass explored for the uptake of heavy metal ions includes maize leaf (Ahalya et al., 2003), tea waste and coffee (Orhan and Buyukgungor, 1993), maple (Bin, 1995), modified sunflower stalk (Hashem et al., 2006), red fir (Bryant et al., 1992), hazelnut shells (Cimino et al., 2000; Dakiky and Khamis, 2002; Demirbas, 2003), saw dusts (Ajmal et al., 1998; Zarraa, 1995), palm kernel husk (Omgbu and Iweanya, 1990), pines bark (Vazquez et al., 1987; Freer et al., 1989; Vazquez et al., 1994; Vazquez et al., 2002), peanut hull (Johnson et al., 2002), diverse bark samples (Al-Asheh and Duvnjak, 1997; Aoyama et al., 1993; Deshkar et al., 1990; Gaballah and Kilbertus, 1998; Gloaguen and Morvan, 1997; Kmiecik et al., 2005; Kumar and Dara, 1980; Kumar and Dara, 1982; Seki et al., 1997; Deshkar and Dara, 1988), apple wastes (Maranon and Sastre, 1991), coconut husk (Tan et al., 1993; Babarinde, 2002), modified cellulosic materials (Shukla and Sakhardande, 1990; Acemioğlu and Alma, 2001), tea leaves (Tee and Khan, 1988), corncobs (Hawrhorne-Costa et al., 1995), modified corncob (Vaughan et al., 2001), rice hulls (Low et al., 1999), banana and orange peels (Annadurai et al., 2002), coffee grounds (Macchi et al., 1986), wool fibers (Balkose and Baltacioglu, 1992), modified lignin (Demirbas, 2005; Demirbas, 2007), modified sugar beet pulp (Reddad et al., 2002), palm fruit bunch (Nassar, 1997), different agricultural by-products (Marshall and Champagne, 1995; Hashem et al., 2006 a, b; Hashem et al., 2007a, b; Chong and Volesky, 2000; Schneider et al., 2001), and wool, olive cake, pine needles, almond shells, cactus leaves, charcoal (Dakiky et al., 2002).

With emerging developments, microbial biomass presents an alternative, ecofriendly and economic (Ting et al. 1988) option that can effectively sequester metal(s) by the process of biosorption (McHale et al., 1994; Gadd et al., 1988; Macaskie et al., 1985; Tsezos and Volesky,

1982). Biosorption is a preferred method due to multiple advantages like low operating cost, easiness, effectiveness with minimum or no sludge production, also effective for industrial wastewater management (Volesky & Holan, 1995). These advantages are pivotal in promoting biosorption as a viable clean-up technology for heavy metal(s) pollution (Kratochivil et al., 1998). The binding capacity of certain biomass is comparable with the commercial resins (Wase and Foster, 1997) AlgaSorb, AMT-Bioclain and Bio-fix are few commercially available microbial biosorbents.

1.3. Microorganisms as Adsorbent

The fate of metals released into aquatic and terrestrial environments due to industrial activities is determined by abiotic parameters. Abiotic factors such as pH, ionic strength, water hardness and the presence of natural organic matter (humic acid) alters the rate of metal erosion (dissolution) and aggregation with naturally occurring colloidal species. On the other hand microbiological activity has also significant influence upon the environmental fate of these pollutants. Almost every food chain has microorganisms at the beginning or at end and their role is foremost in more or less all biogeochemical cycles. Green and Darnel (1990) described the removal and recovery of metals by microorganisms. Some microbes express preference for certain heavy metals and few others are broad range (Hosea et al., 1986; Volesky et al., 1988). The major groups of microorganisms drawn in comprise bacteria, cyanobacteria, microalgae and fungi (Gadd, 1996). These groups are explored by virtue of their close connection with the surrounding metals in the effluent and slurries. Volesky and Holan have presented a comprehensive list of microbes and their adsorption extent for several heavy metals.

Marine algae a photoautotroph is a candidate of interest due to its bulk availability. Kuyucak and Volesky (1989 a, b, c) reported high biosorption capacities of *Sargassum natans* and *Ascophyllum nodosum* for various metals. Besides marine algae, *Chlorella sp.* of green algae is reported by Khummongkol et al., (1982), Ting et al., (1991) and Aksu and Kutsal, (1990). Among bacteria, reports on the *Bacillus sp*, *Pseudomonas sp*, *Zoogloea ramigera* and *Streptomyces sp.* are of great importance (Strandberg et al., 1981; Nakajima and Sakaguchi, 1986; Mullen, 1989; Norberg and Persson, 1984; Norberg and Rydin, 1984). The sorption capacity of cyanobacteria is also assessed by Horikoshi and Nakajima (1979; 1981).

Omnipresence and intracellular metal uptake capacity of fungi provided momentum for metal ions remediation from wastewater than other microbes. The great tolerance towards heavy metals and other adverse abiotic factors, suggest them appropriate substitute (Gadd, 1987). Among the groups of fungi, *basidiomycetes* (wood rotting fungi) easy cultivation, high yield and safety have been known from decades. Therefore, biosorbents made from mycelial biomass of these fungi could be easily applied practically (Veit et al., 2005; Jarosz-Wilkolazka et al., 2006; Gonen et al., 2008; Vimala & Das, 2009). Fungi another new family of biosorbent is under wide exploration using byproducts from large-scale fermenters (Niu et al., 1993; Paknikar et al., 1993; Nemeč et al., 1977). Brewer's yeast great accomplishment is elaborated by a number of researchers (Gadd and White, 1993; Brady et al., 1994; Brady and Duncan, 1994; Tobin et al., 1990; Volesky and Holan, 1995; Wilhelmi and Duncan, 1995; Unz and Shuttleworth, 1996; Riordan et al., 1997; Bakkaloglu et al., 1998). Excellent potential of metal biosorption by fungi and yeast is noted in particular for genera *Rhizopus*, *Aspergillus*, *Streptovercillum* and *Saccharomyces* (Volesky and Tsezos, 1981; Galun et al., 1984; DeRome and Gadd, 1987; Luef et al., 1991; Brady and Duncan, 1993).

Metal pollutants can be bound or precipitated by microbial products and metabolites, accumulated by cells through non-specific physico-chemical interactions as well as specific mechanisms of sequestration or transport and oxidation-reduction reactions.

Norris et al., (1979) elaborated two distinct processes for cobalt and cadmium accumulation by *Saccharomyces cerevisiae*. Initially, rapid binding of cation is metabolism and temperature independent stage (Passive biosorption). It is followed by much slower metabolism dependent cation internalization process (active biosorption). Intracellular active process of metal remediation is called bioaccumulation mediated only by living biomass. This second process is believed to accumulate larger quantities of cation into the cell than first one (Norris et al., 1977). Jianlong and Can (2006) discussed unique nature and metal sorption capacity of *S.cerevisiae*. Marmeeva and Podgorsky (2009) described it as one of the most promising biosorbents for chrome Cr (VI) from aqueous solutions. Huang et al. (1988) used *cerevisiae* to remove copper, zinc, lead and cobalt.

Among microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties (Rosenberger, 1975; Gadd 1990; Paknikar et al., 1993). Heavy metals biosorption on different materials such as red algae (Sariet

al., 2008), moss lichen (Uluzlu, 2008), yeast biomass (Tonk et al., 2011), tea waste (Cay et al., 2004), various type of sawdust (Costodes et al., 2003; Chakravarty et al., 2010) is intensively investigated. Yeasts are found gifted for capturing cations such as copper, nickel and manganese, and expressed as better metal accumulators than certain bacteria (Norris et al., 1979).

The ability of wood rotting fungi to adsorb metals is same or even better, than that of lower fungi and yeast (Javaid, 2008). Amna et al., (2010) reported 18.54 mg/g biosorption capacity of *Schizophyllum commune Fries*, for Cr(VI) which is a wood rotting fungus. The excellent mechanical properties of fungal mycelia provide an opportunity to utilize them for selective sorption of heavy metal ions (Bayramoglu et al., 2005; Razmovski & Šćiban, 2008). These materials proved to be both green and eco-friendly materials. Several mushroom biomasses, *Lentinus edodes* (Chen et al., 2008), *Amanita rubescens* (Sari et al., 2009), *Pleurotus platypus*, *Agaricus bisporus* (Vimala et al., 2009), *Pycnoporus sanguineus* (Mashitah et al., 2008), *Lactarius scrobiculatus* (Anayurt et al., 2009) are also proven to be good alternative biosorbents for the removal of metal ions (Vimala and Das, 2011; Fu and Wang, 2011) from wastewater.

With all advantages, the application of biosorption process in practice is indeed difficult (Tsezos, 2001). Studies on biosorption have demonstrated that some cultivated mushrooms can bioaccumulate considerable amount of metal ions. However, quite a few researchers report that fruit bodies of mushrooms accumulate remarkably high concentrations of Cu and Zn (Sugahara et al., 1990; Vetter et al., 1990; Vetter et al., 1994; Vetter et al., 1997; Falandysz, 1993; Jorhem, 1995; Pop & Nicoara, 1996; Sesli et al., 1999). This indicates the potential use of mushrooms as effective adsorbents for heavy metals.

Pleurotus ostreatus is the most common edible wild cultivated mushroom. It is chosen in this study to serve as biosorbent for metal pollution because its biomass is cheap and easily available. Also its pure cultures and fruiting bodies are available at research institutes and at mushroom farms.

1.4. Biomass Engineering for Metals Uptake

The natural biomass materials offer good adsorptive properties. The diverse and reactive groups as hydroxyl, carboxyl, carbonyl, thiol, phosphonate, amine, amide, etc. (Sheng et al., 2004) on biomass act as ion exchangers for metals. Furthermore, more metal binding sites can be created through pretreatments of a biosorbent making the adsorption process more economical. The pretreatments modify the surface characteristics/ groups either by removing or masking the groups

or by exposing binding sites (Paknikaret et al., 1993). For instance, the presence of amine groups on the structure is suggested to interact physically and chemically with other species, and they are highly sensitive to pH changes (Gadd et al., 1993; Baldrian, 2003).

1.5. Immobilized Biomass for Industrial Applications

Immobilization techniques are significant to retain the ability of microbial biomass to sorb metals during the continuous industrial processes (Golab et al., 1991; Ross et al., 1986). The excessive hydrostatic pressures cause disintegration of free cells due to their low mechanical strength and small particle size. Immobilization (Gadd, 1990; Leusch et al., 1995) provides better reusability, high biomass loading and minimal clogging of biomass in continuous flow systems (Holan & Volesky, 1994; Gourdon et al., 1990).

A number of matrices have been employed for immobilization of cells. The entrapment in the matrix of insoluble Ca-alginate (Cotoraset al., 1993) has been used in binding of cobalt, zinc and manganese (Volesky, 1994). Silica is another important immobilization matrix and the products obtained are mechanically strong and exhibits excellent flow characteristics (Beveridge and Fyfe, 1985). Further, inert solid supports like polyvinyl chloride, ZirFon R membrane, glass, metal sheets, plastics, uneven surfaces are used as biofilms (Macaskie & Dean, Kuhn & Pfister 1989) for the immobilization of biomasses.

1.6. Development of Biocomposites

Dynamic development of biocomposites has been driven by the need to obtain products of specific unique properties. For instance, Chitin as an adsorbent bed has low mechanical and chemical resistances compared to typical inorganic materials as silica. On the other hand, Chitin bentonite composite act as effective adsorbent to acidic environment (Veera et al., 2008) for removing metals.

The biocomposites are homogeneous, porous and mechanically resistant in real scale adsorption processes. The development of composites is a simple method to support or reinforce materials.

The production of SiO₂ composites with organic biopolymers has recently gained much attention. Silica is attractive for the majority of new products of different morphologies, because of its large surface area and high mechanical strength. An additional advantage of SiO₂ is the possibility of its surface modification to change its chemical and physical properties to meet particular demands

(Jesionowski et al., 2003). The presence of many functional oxygen groups on silica surface enables its modification accompanied by formation of more active sites, which increases its ability to adsorb heavy metal ions, organic compounds and many other pollutants (Hayashi et al., 1997; Kajiwara & Chujo, 2011; Qu et al., 2010).

The use of lignin as a biodegradable polymer in an inorganic matrix (such as silica, alumina) permits obtaining a highly functional material. High abundance and natural character of lignin in combination with silica makes this material an excellent polymer filler permitting getting products of desired properties at low cost (Ignat et al., 2011; Stiubianu et al., 2009).

So far rare attempts at obtaining such inorganic-organic products have been made. In view of the novel industrial potential applications, it is important to determine substrate for synthesis of hybrid materials and development of biocomposites.

1.7. Objectives of the Present Study

The present study is designed to investigate the sorption capacity of the selected Mushroom species for the toxic metals. Further, relationship between chemical constituents and adsorption phenomenon is also attempted through detailed characterization and batch adsorption experiments. The metal remediation model is also proposed by preparing a wide range of adsorbents. For this purpose, biocomposites of Macrofungus with inorganic moieties of silica and alumina are prepared. In another series, Macrofungus is functionalized to enhance the efficiency and then combined with silica and alumina. It is important to mention that both inorganic precursors are known as good adsorbents with hydroscopic characteristics.

The specific objectives are defined as:

- Exploring the capacity of Macrofungus as biosorbent in metals removal under different pH conditions.
- The applicative technological development of biocomposites under optimization of pH for biosorption of metals
- Proposing an equilibrium model based on experimental data of metal adsorption studies to develop mechanistic understanding of the adsorption process.

- The optimization of the operative conditions and development of immobilized and stable organic-inorganic composites for the design of industrial continuous processes.

1.8. Significance of the Study

The present study is significant in developing biosorbents with improvised characteristics like immobilization of the Macrofungus. Immobilization of the biomass can bestow ideal size, mechanical strength, rigidity and porous characteristics to the biological material (Brierley and Brierley, 1993). Natural polymers such as alginate, chitosan, polyvinyl alcohol are commonly used matrix for immobilization of the biomass and enhance biosorption for the removal of heavy metals due to mechanical and chemical stability.

In the present investigation, the development of an economically viable immobilizing protocol of Macrofungus with its sorptive properties preserved is significant. Further, cell surface of Macrofungus is functionalized with calcium (divalent) alginate rather than conventional sodium (monovalent) alginate (Xiangliang et al. 2005). It is expected that immobilized biomass will offer more binding due to lower ionic radius of Ca^{+2} ions.

The optimization of environmental conditions like pH and combination of Macrofungus with silica and alumina into biocomposite beads adds the advantage of stability and simultaneous sorption and desorption process. The economical viability of commercialization for sustainable development of these biocomposites as adsorbents is another important perspective.

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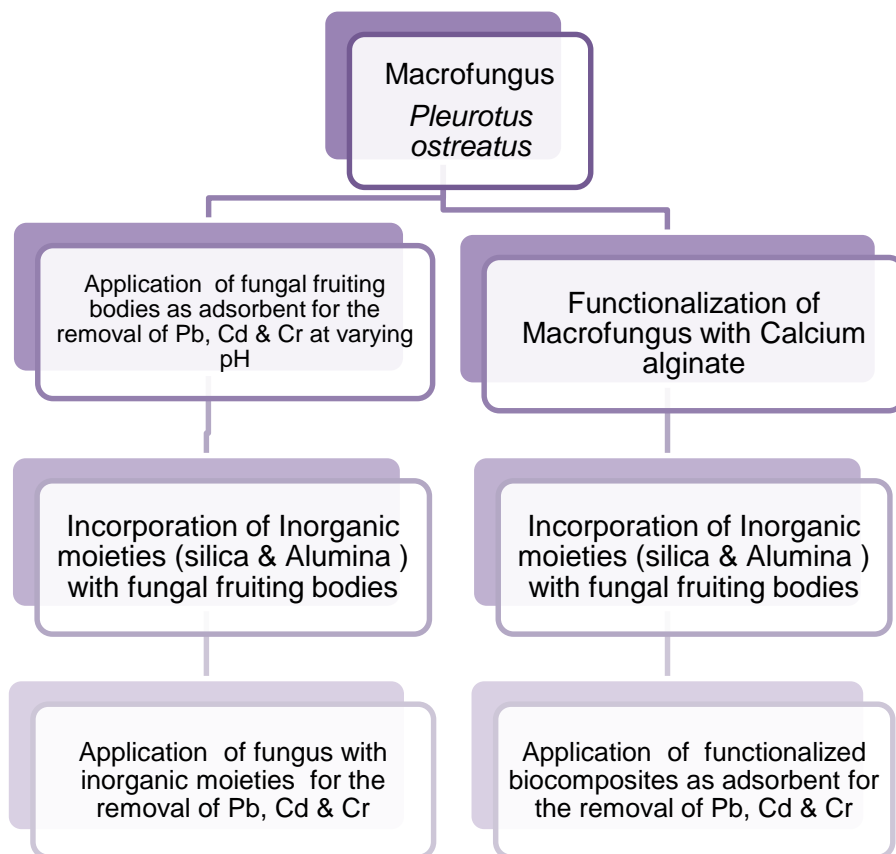
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CHAPTER 2 - EXPERIMENTAL

The present research is directed at the utilization of macro-fungus for the development of bio composite to be further applied as adsorbent for the removal of toxic metals. The selected Macrofungus is *Pleurotus ostreatus*. For this purpose, two series of adsorbents were prepared. Series A comprise of Macrofungus fruiting bodies (MF), Macrofungus Silica composite (MFSi), Macrofungus alumina composite (MFAI), Series B consist of Functionalized Macrofungus (FMF), Functionalized Macrofungus Silica composite (FMFSi), Functionalized Macrofungus Alumina composite (FMFAI), and functionalized Macrofungus silica-alumina hybrid (FMFSiAl).

Each adsorbent is applied in batch mode for the removal of Pb, Cd and Cr. The adsorbed concentration was analyzed on Flame Atomic Absorption Spectrophotometer. The Flowchart for Experimental work is given below:



2.1.Pre-treatment of Macrofungus

Fruiting bodies of *Pleurotus ostreatus* (Macrofungus) were obtained from Choudhry Mushroom Farms Wah Cantt. Fruiting bodies were washed repeatedly with distilled water to remove debris. The Macrofungus was dried in the folds of blotting sheets followed by oven drying at 105° C till constant weight is attained. The dried biomass was ground to powder and sieved through US Standard mesh of 100 to obtain particle sizes of 180-150µm, respectively.

Pleurotus ostreatus is a commonly available and economical source of biomass. It also adds novelty to present research that limited or no study is carried out on the development of composites using Macrofungus with inorganic precursors.

2.2.Preparation of Bio-composites

The present study reports the development of composites based on the selected Macrofungus with inorganic moiety of silica and alumina. Functionalization of Macrofungus with basic solution of calcium alginate is another novel attempt. The general procedure is detailed as follows.

2.2.1. Preparation of Macrofungus based biocomposites (Series A)

Two composites of Macrofungus with silica and alumina are prepared. For this purpose, pre-treated Macrofungus is agitated with silica (Analytical Reagent Grade, Merck) in a ratio of 10:1 (w/w), respectively. The mixture was homogenized in agate pestle mortar till intimate physical contact between the two components is merged into a biocomposite. Then water is added to the mixture and subjected to stirring for 1 hour. The mixture is left for overnight and extruded through syringe for bead formation (Klapizewski et al., 2013). The product obtained is coded as MFSi.

The same procedure is adapted for the preparation of Macrofungus based alumina composite. The product is coded as MFAI.

2.2.2. Preparation of Functionalized Macrofungus based biocomposites (Series B)

Three composites of Series B are prepared using Calcium alginate for the functionalization following the protocol of (Wang et al., 2005) with slight modification. The general procedure is as follows:

Stock solution of calcium alginate in slightly alkaline aqueous medium is prepared. A known volume of this solution is added to a known mass of Macrofungus with continuous agitation for

complete dissolution to yield functionalized Macrofungus(FMF). The functionalized Macrofungus composites with inorganic moiety (silica and alumina) are prepared by homogenizing calcium alginate, Macrofungus and inorganic precursor in a ratio of (1:10:1), respectively. The functionalized composites are coded as FMFSi and FMFAI. A hybrid (FMFSiAl) of both inorganic moieties with stabilized Macrofungus is developed by taking equimolar quantities. Each of the products is extruded through 10 ml syringe for bead formation. The beads are rinsed with deionized water and allowed to dry at room temperature before further use. Prepared biocomposites with specification and respective groups are listed in Table 2.1.

Table 2.1:Sample Specification and Sample Codes of Macrofungus based Biocomposites

Sample Codes	Sample Specification
Pristine Samples	
MF	Macrofungus (<i>Pleurotus ostreatus</i>)
Si	Silica(SiO ₂) AR Grade (pristine)
Al	Alumina (Al ₂ O ₃) AR Grade (pristine)
CA	Calcium Alginate(C ₁₂ H ₁₄ CaO ₁₂) _n AR Grade
Non-Functionalized Biocomposites	
MFSi	Composite of Silica with Macrofungus
MFAI	Composite of Alumina with Macrofungus
Functionalized Biocomposites	
FMF	Composite of Macrofungus with Calcium Alginate (Functionalized Macrofungus)
FMFSi	Composite of Silica with Functionalized Macrofungus
FMFAI	Composite of Alumina with Functionalized Macrofungus
FMFSiAl	Silica Alumina hybrid with Functionalized Macrofungus

2.3. Determination of Active Sites by Boehm's Titration Method

Basic and acidic active sites on the surface of *P. ostreatus* fruiting bodies and its composites with organic and inorganic moieties is estimated following Boehm's (2008) titration procedure.

For this purpose, three reaction bases (NaHCO₃, Na₂CO₃ and NaOH) are selected. Each base is added separately to 1.0 g of sorbent and agitated on shaker at 100 rpm for 24 hours at ambient temperature. The solution is filtered to remove the sorbent.

Base aliquot is titrated in triplicate against 0.05 M HCl for complete neutralization in 1:1.5 ratios for monoprotic and diprotic bases, respectively using phenolphthalein and methyl red as indicator. Blank sample is run for each reaction base. The acidified solution is back-titrated in triplicate with 0.05 M NaOH.

Acidic and basic surface groups (Chen & Wu, 2004) by back titration are determined using the equations (a) and (b) respectively.

$$[\text{HCl}]V_{\text{HCl}} = [\text{NaOH}]V_{\text{NaOH}} + \left(\frac{n_{\text{HCl}}}{n_{\text{B}}} [\text{B}]V_{\text{B}} - n_{\text{CSF}} \right) \frac{V_{\text{A}}}{V_{\text{B}}} \quad (\text{a})$$

$$n_{\text{CSF}} = \frac{n_{\text{HCl}}}{n_{\text{B}}} [\text{B}]V_{\text{B}} - ([\text{HCl}]V_{\text{HCl}} - [\text{NaOH}]V_{\text{NaOH}}) \frac{V_{\text{B}}}{V_{\text{A}}} \quad (\text{b})$$

The molar ratio of acid to base (Sarah et al., 2010) for monoprotic versus diprotic reaction bases is computed using equation (c)

$$n_{\text{CSF}} = \frac{n_{\text{NaOH}}}{n_{\text{A}}} [\text{A}]V_{\text{A}} - ([\text{NaOH}]V_{\text{NaOH}} - [\text{HCl}]V_{\text{HCl}}) \frac{V_{\text{A}}}{V_{\text{B}}} \quad (\text{c})$$

2.4. Characterization of Biocomposites

Each of the developed biocomposite is comprehensively characterized by a wide range of techniques to determine the surface and bulk characteristics.

2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 400 cm⁻¹.

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum.

The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

In the present investigation, FTIR spectra are recorded on Fourier Transform Infrared spectrophotometer, SHIMADZU 8400, Japan as pressed KBr pellets under 60 KN of pressure. For this purpose, primarily, the Macrofungus and its composites are analyzed to determine the characteristic functional groups present in pristine samples.

Secondly, the prepared biocomposites loaded individually with Pb, Cd, and Cr are subjected to FTIR to investigate the linking groups of metal to composites.

2.4.2. Scanning Electron Microscopy (SEM)

The surface morphologies of the samples are determined under Scanning Electron Microscope. The technique uses a general principle of impinging a beam of electrons emitted by a filament and scanning across the surface of the sample. The electrons upon interaction with the atoms of the sample produces signals of secondary electrons (SE), back-scattered electrons (BSE), characteristic X-rays, which contain information about the sample's surface topography and composition. The detectors collect the required signals and convert them to a signal to a viewing screen. This method when combined with energy dispersive X-ray microanalysis (EDX) analyzes the secondary and back-scattered electrons and gives information about the elemental composition of the sample. For the present research, SEM images of Macrofungus based biocomposites are recorded on Scanning Electron Microscope, at an acceleration voltage of 4 kV with gold sputtered coating.

2.4.3. BET Surface Area Analysis

The measurement of the specific surface area of a material can conveniently be determined by BET nitrogen gas adsorption method. BET theory (proposed in 1938) explains the surface area on the basis of physical adsorption of gas molecules on a solid surface. This concept is an extension of Langmuir theory for monolayer to multilayer adsorption.

The Brunauer-Emmett-Teller (BET) equation (Do, 1998) is as follows:

$$\frac{p}{q(p_s - p)} = \frac{1}{q_m c} + \frac{c - 1}{q_m c} \left[\frac{p}{p_s} \right]$$

Where, p defines operating pressure; p_s saturation vapor pressure and c is operational constant. P/P_s is plotted against 'q' to determine the BET parameters.

The specific surface area, pore size and pore volume of the samples were measured using N₂ gas sorption. Nitrogen adsorption-desorption measurement was performed at 77K using a Micromeritics ASAP 2020 Porosimeter Test Station.

2.5. Development of Biocomposites as Adsorbents

The prepared composites of both series are developed as adsorbents for the removal of toxic metals from aqueous media. The dried biomass was ground to powder and sieved through US Standard mesh of 100 to obtain particle sizes of 180-150 μm, respectively. Size of the beads measured with screw gauge is 1.30-1.38 mm.

2.5.1. Batch Adsorption Protocol

Batch adsorption experiments were performed by taking Xgms (50 mg) of biosorbent in 200ml metal solution (Pb, Cd & Cr). The adsorption studies were done as a function of pH (2, 3, 3.5, 4, 4.5, 5, 6, 7) and contact time (0 min to 45 min) with a three minutes time interval. The contact time was predetermined by performing batch experiments of varying time duration and intervals.

Aliquots of 10ml were taken and filtered through Whatman filter paper. 40 and residual metal concentration was determined through AAS.

The amount adsorbed per unit mass of the adsorbent (q_e) was plotted against time.

Metal removal (%R) was calculated using the equation:

$$\%R = \left(\frac{C_i - C_t}{C_i} \right) 100$$

Sorption efficiency q_e (mg/g) was determined as:

$$q_e = \left(\frac{C_i - C_e}{W} \right) V$$

where C_i, C_t and C_e (mg/L) are the liquid- phase concentrations of adsorbate initially, at time t and at equilibrium, respectively. V is the volume (L) of the solution and W is the weight (g/L) of sorbent.

Distribution Ratio (K_d) is calculated from the following relation:

$$K_d = \frac{\text{amount of adsorbate in adsorbent}}{\text{amount of adsorbate in solution}} \times \frac{1}{S}$$

2.5.2. Equilibrium and Kinetic Studies

The data set obtained from the experimental design of the present investigation was treated with widely accepted adsorption Isotherms and kinetics models. The objective was to determine the efficiency of the fungal biocomposite based adsorbents for the remediation of different pollutants. The Isotherms quantifies the ability in terms of the amount of pollutant adsorbed per unit mass of the adsorbent and adsorption kinetics determines the rate of removal.

Linearized form of isotherms and kinetic models and their parameters are listed in Table 2.2.

Table 2.2: Linear Expression for Adsorption Isotherms and Kinetic Models

<i>Linear expression</i>	<i>Plot</i>	<i>Parameters</i>
Adsorption Isotherms		
Langmuir (1918)	$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m}$	C_e / q_e v C_e $q_m = 1 / \text{slope}$ $K_L = \text{slope} / \text{intercept}$
Freundlich (1906)	$\log q_e = \log K_F + \frac{1}{n} \log C_e$	$\log q_e$ v $\log C_e$ $n = 1 / \text{slope}$ $K_F = \text{Antilog}(\text{intercept})$
Temkin (1940)	$q_e = B \ln K_T + B \ln C_e$	q_e v $\ln C_e$ $B = \text{slope}$ $K_T = \exp(\text{intercept} / \text{slope})$
Kinetic Models		
Zero order	$(q_e - q_t) = q_e - kt$	$(q_e - q_t)$ v t $k = \text{slope}$ $q_e = \text{intercept}$
Pseudo 1st order	$\log(q_e - q_t) = \log q_e - \left(\frac{k_1}{2.303}\right)t$	$\log(q_e - q_t)$ v t $k_1 = \text{slope}$ $q_e = \text{Antilog}(\text{intercept})$
Pseudo 2nd order	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right)t$	$\frac{t}{q_t}$ v t $q_e = \text{slope}$ $h = \text{intercept}$ $k_2 = \text{intercept} / (\text{slope})^2$
Intra particle diffusion	$q_t = k_{ip} t^{0.5} + C$	q_t v t $k_{ip} = \text{slope}$ $C = \text{intercept}$

CHAPTER 3 - RESULTS AND DISCUSSION

The present research is based on the development of bio composite from Macrofungus using inorganic moieties and calcium alginate. Each biocomposite is further applied as adsorbent for the removal of toxic metals (Pb, Cd and Cr). The adsorbed concentration is analyzed on Flame Atomic Absorption Spectrophotometer.

Each of the prepared fungal biocomposite is comprehensively characterized by a range of techniques to determine the surface and bulk characteristics. The characteristics revealed by each technique are highlighted in the following section:

3.1.FTIR Analysis

The pressed KBr pellets of prepared samples are analyzed on FTIR for identification of surface functional groups. The important frequencies marked on the spectra are given in Figures 3.1-3.5. The dried Macrofungus (MF) as raw material of composites indicates clearly a broad band at 3404 cm^{-1} assigned to -OH and -NH stretching (see Figure 3.1-3.3a). This is attributed to the presence of glucose and amino protein as natural constituent of fruiting bodies. The stretching of -CH at 2922 cm^{-1} , C-O-C at 1043 cm^{-1} , C=O conjugation and -NH deformation of amide I at 1654 cm^{-1} all contribute to peptide bond.

This suggests that basic framework of biomass is constructed on peptide linkage as discussed later in EDX analysis.

3.1.1. FTIR of Macrofungus Composites (without Metal Loading)

The FTIR is also recorded for neat samples of silica and alumina of analytical reagent (AR) grade. Silica sample shows three absorption bands at 1087 , 800 and 939 cm^{-1} (see Figure 3.1b) which is due to the presence of Si-O , and Si-OH bond of SiO_2 . Further, Si-O-Si bonds vibrational modes are detected around 484 , 800 and 1087 cm^{-1} which are attributed to O-Si-O rocking vibration band (Ryu, 2006), Si-O bending vibration band and Si-O-Si anti-symmetric stretching vibration band, respectively (Rassy and Pierre, 2005; Innocenzi et al., 2003).

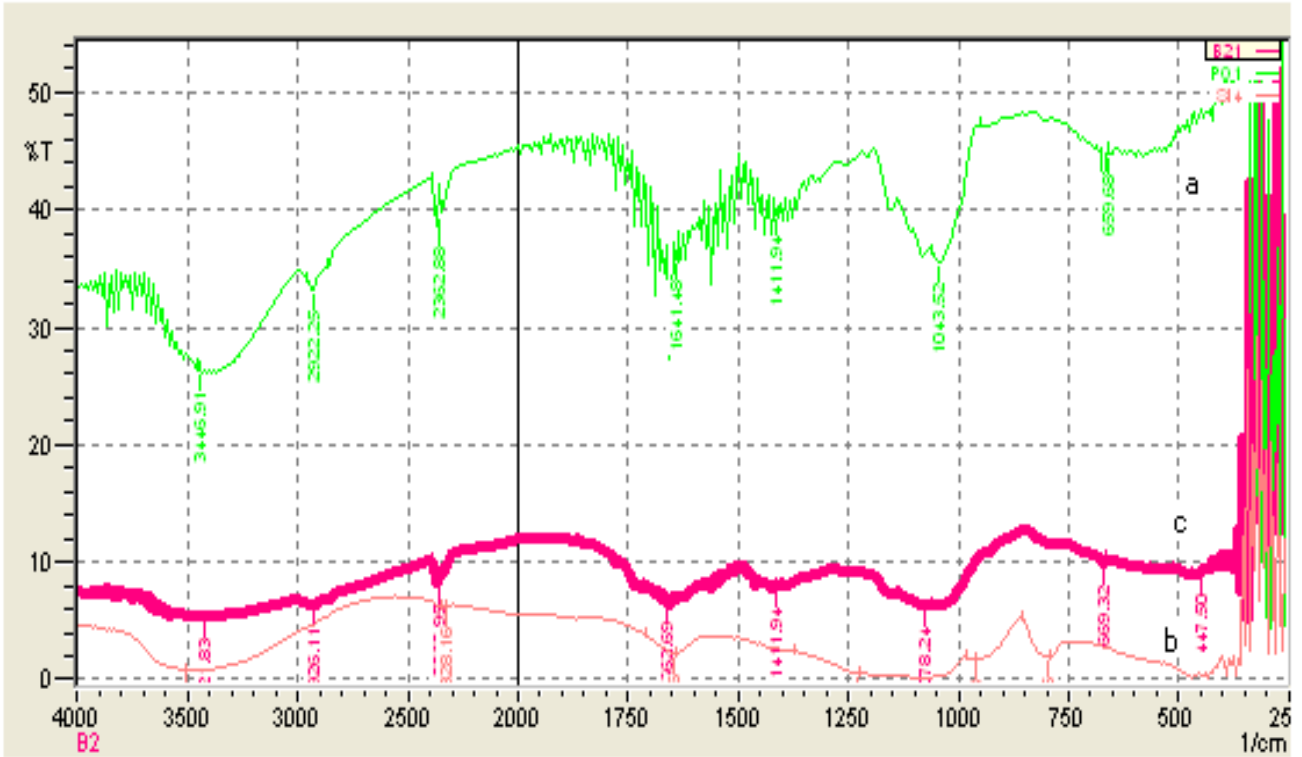


Figure 3.1 FTIR spectra of Silica Composite with Macrofungus (a) MF (b) Silica (c) MFSi (composite)

On the other hand, a narrow band at 613 cm^{-1} is attributed to Al–O stretching vibrations in alumina (see Figure 3.2b). The detection of band at 1114.89 cm^{-1} with a shoulder at 1158 cm^{-1} , is attributed to symmetrical and anti-symmetrical Al–OH bending modes, and Al–O bending vibration in aluminum octahedral (Al_2O_3) units. The absorption band at 1624 cm^{-1} is due to moisture in the sample (Chih-Peng and Shaw-Bing, 2002).

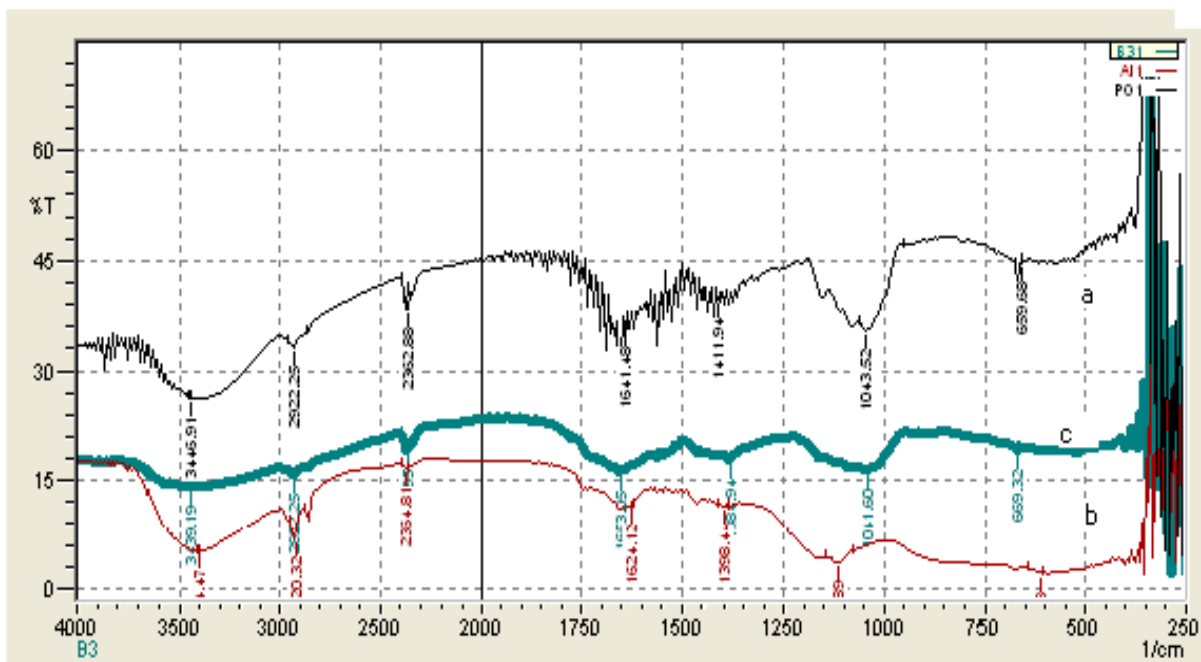


Figure 3.2 FTIR spectra of Alumina Composite with Macrofungus (a) MF (b) Al (c) MFAI (composite)

The pristine sample of Calcium alginate (procured from Merck) is pressed as pellet and analyzed under FTIR spectrophotometer. The spectra (Figure 3.3b) shows one broad absorption bands at 3404 cm^{-1} due to stretching of $-\text{OH}$, and two sharp bands at 1637 cm^{-1} and 1419 cm^{-1} are assigned to $-\text{COO}$ asymmetric and symmetric stretching indicating the presence of alginic acid. The band at 1031 cm^{-1} with shoulders indicate $-\text{C}-\text{O}$ stretching of ether and alcoholic groups.

The functionalization of Macrofungus is an important parameter. For this purpose, fruiting bodies are immersed in the alkaline calcium alginate solution. The resultant dried mixture is pressed into pellet for FTIR analysis. The spectra (see Figure 3.3 c) reveals interesting modifications. It is noted that $-\text{CH}$ stretching at 2922 cm^{-1} in pristine samples of calcium alginate and Macrofungus broadens in the functionalized sample. It suggests that binding is facilitated through $-\text{CH}$ linkage. In addition, new peaks emerge at 1705 cm^{-1} , 1500 cm^{-1} , and 1205 cm^{-1} , indicating the successful impregnation of calcium alginate onto surface of Macrofungus.

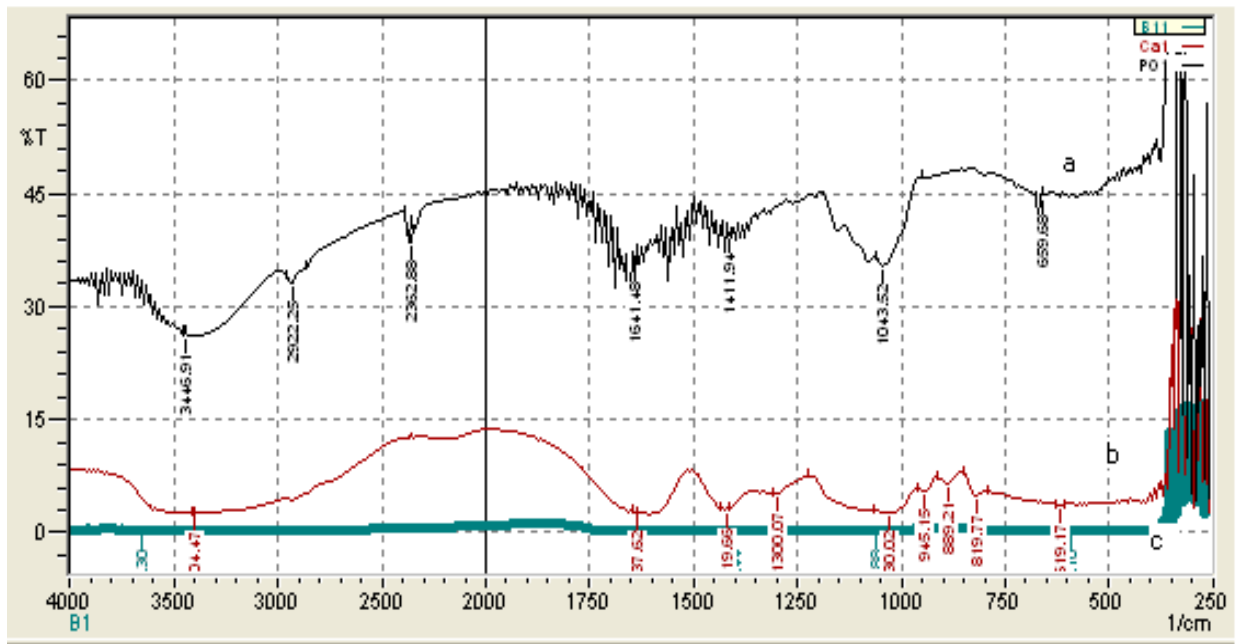


Figure 3.3: FTIR Spectra of Functionalized Macrofungus a. MF b. Calcium Alginate c. FMF

The extrusion of aqueous paste of Si-and Al based composite of Macrofungus into beads is also subjected to FTIR analysis. The spectra of two biocomposites are represented in Figure 3.1 (c) and 3.2(c). It is encouraging to note the complete disappearance of unreacted SiO₂ showing peak at 939 cm⁻¹ and shift of wavenumber at 484 cm⁻¹ to 447 cm⁻¹ clearly indicates the composite formation of silica with Macrofungus. Similarly, the linkage of alumina with Macrofungus is attributed to shift of Al–O stretching vibrations in pristine Alumina to higher wave number (669 cm⁻¹) in the composite (see Figure 3.2(c)). The Ca-alginate functionalized Macrofungus is also attempted for composite formation with silica and alumina.

The assessment of change in functional groups is predicted in FTIR spectra shown in Figure 3.4(a-c). The similarity and dissimilarity to the Macrofungus and functionalized Macrofungus, in respective order, suggests that higher ratio of fruiting bodies contribute more to tri-composite. Further, a significant shift at 542cm⁻¹ to 472 cm⁻¹ (see Figure 3.4b) and 617 cm⁻¹ (see Figure 3.4c) is evidence.

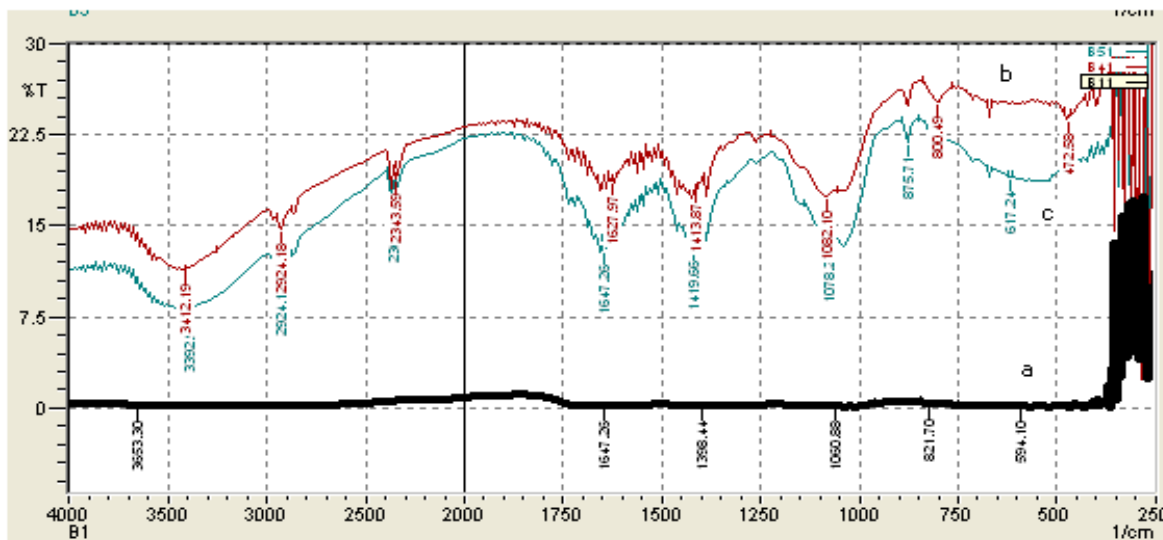


Figure 3.4 FTIR Spectra of Biocomposite of silica and alumina with Functionalized Macrofungus
a. FMF b. FMFSi c. FMFAI

A tri- composite comprising of functionalized Macrofungus, silica and alumina is also analyzed under FTIR. No significant individual contribution of silica and alumina is observed in tri-composite (see Figure 3.5). This might be attributed to the fact that ratio of both remains same in functionalized Macrofungus (1:1:10), respectively.

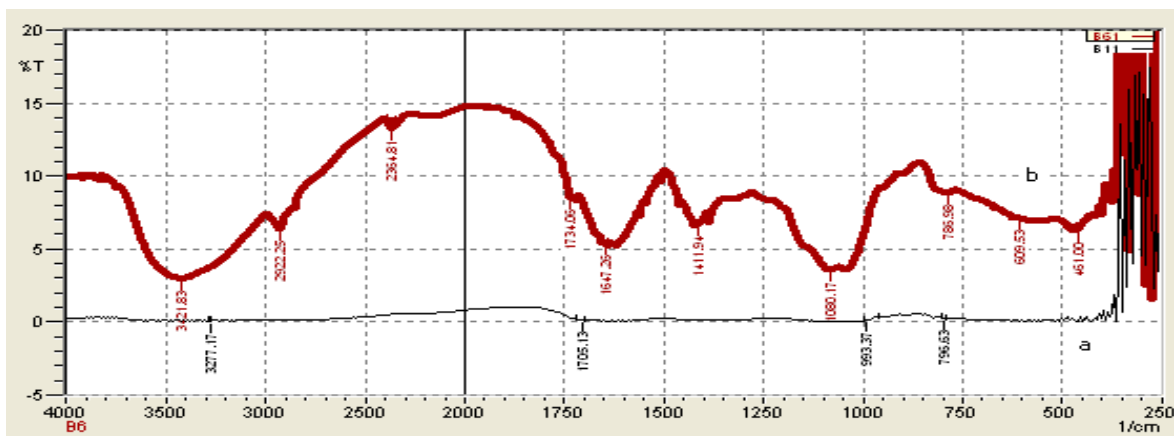


Figure 3.5 FTIR Spectra of Silica Alumina Hybrid with Functionalized Macrofungus
a. FMF b. FMFSiAl

3.1.2. FTIR of Macrofungus Composites (with Metal loading)

The Macrofungus and its composites are induced with known concentration of metal salt solutions of Pb, Cd, and Cr. The spectra are presented in Figure 3.6-3.11(a-c). The Macrofungus and its

composites are induced with known concentration of metal salt solutions of Pb, Cd, and Cr. The spectra are presented in Figure 3.6-3.11(a-c). The loading of Pb onto Macrofungus clearly identify a shift of the broad peak at 3404 cm^{-1} to 3462 cm^{-1} . This suggests that metal bonding is through hydroxyl and amine groups (Arbanah et al., 2013; Xiangliang et al., 2005).

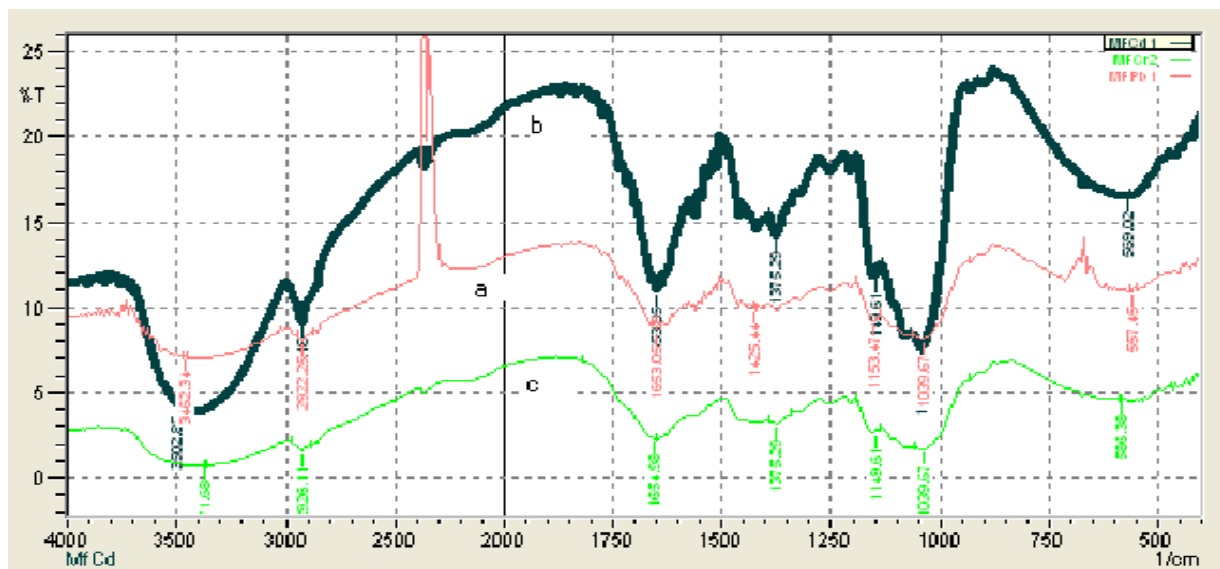


Figure 3.6 FTIR Spectra of Macrofungus loaded with (a)Pb (b)Cd (c)Cr

Similar observations are recorded by (Nagy et al., 2014) for *A. bisporus* species. Their involvement of $-NH$ group is contributed from chitin and chitosan (Javaid et al., 2011). Further, the shift of sharp peak at 1406 cm^{-1} to 1425 cm^{-1} likely attributes the engagement of $C-C$ bond (see Figure 3.6 a).

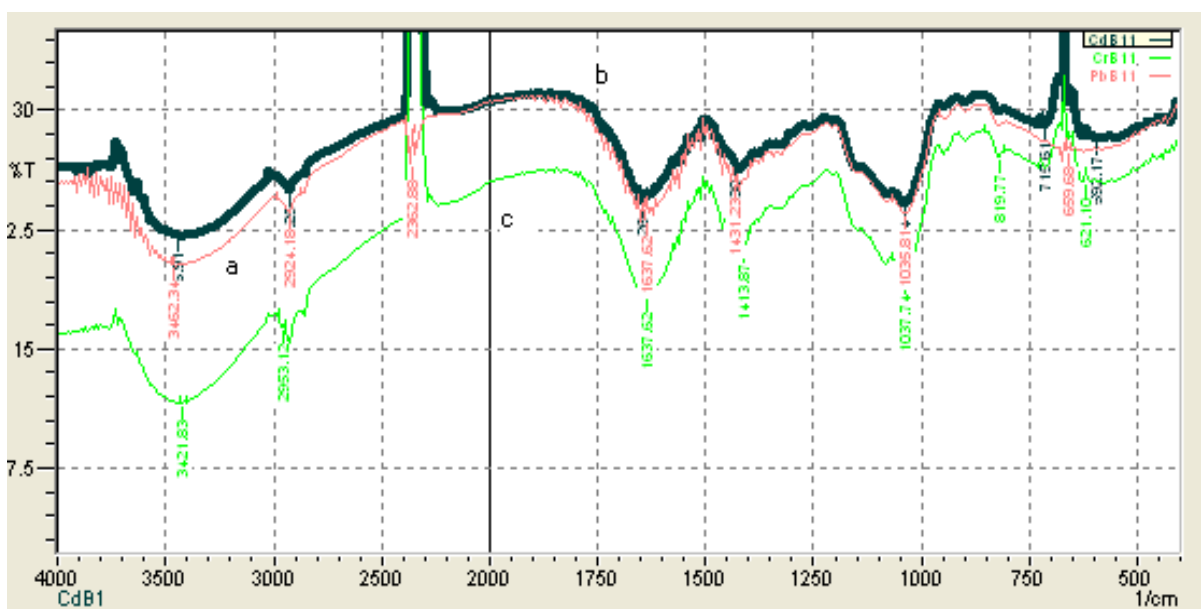


Figure 3.7: FTIR Spectra of Functionalized Macrofungus loaded with (a) Pb (b) Cd (c)Cr

Similarly, Macrofungus loaded with Cadmium (MFCd) and Chromium (MFCr) also demonstrates the shift of 3404cm^{-1} to 3502cm^{-1} and 3371cm^{-1} , respectively. In addition, another shift from 1406cm^{-1} down to 1375cm^{-1} is observed for both Cd and Cr. Similar results are reported for biosorption of Cd (II) on *P. platypus* (Vimala & Das, 2011), *Botrytis cinerea* (Akar & Tunali, 2005), *Pleurotus mutilus* (Bal & Bal, 2006), and *Trametes versicolor* (Subbaiah et al., 2011).

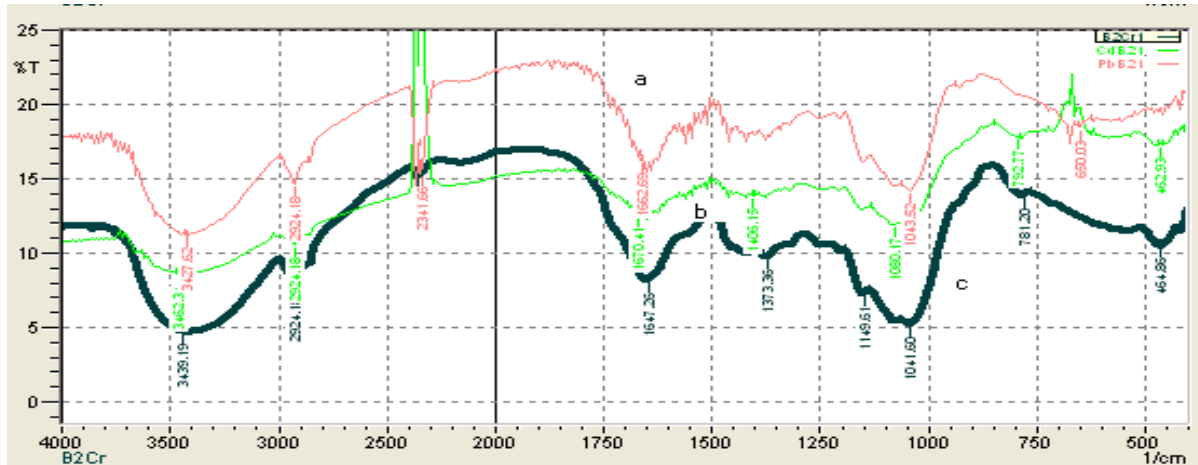


Figure 3.8: FTIR Spectra of Macrofungus Silica Composite (MFSi) loaded with Pb (a), Cd (b) Cr (c)

Contrary to Pb loaded Macrofungus, the emergence of new peak at 1149cm^{-1} is noted for Cd and Cr loaded MF. This may arise due to activity of carboxyl group of protein (Figure 3.6 b-c). The engagement of these functional groups is likely due to the fact that cell wall of fungus is composed of groups carboxyl, phosphate, amide, and hydroxide (Bayramoglu & Arica, 2008).

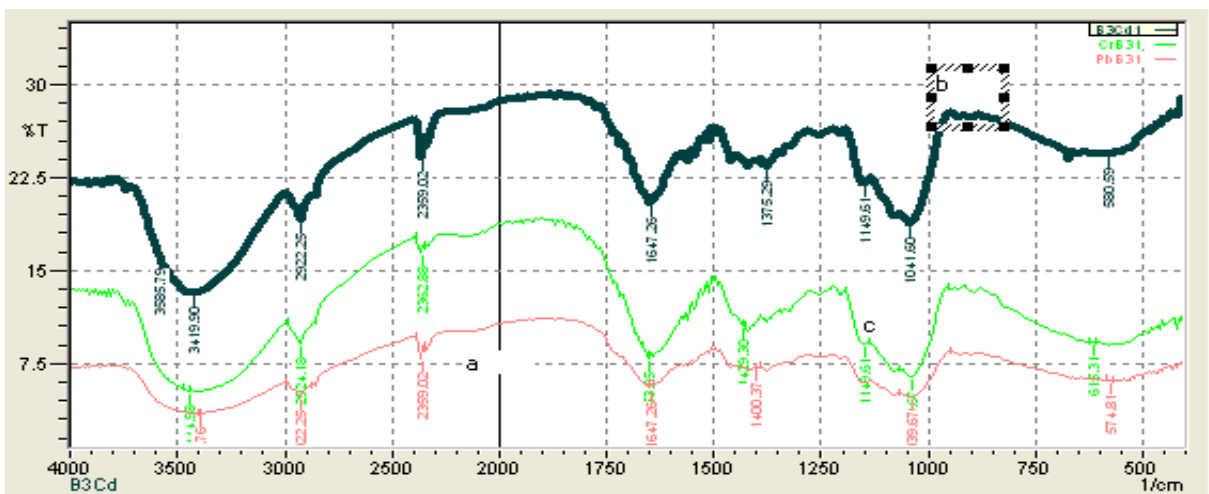


Figure 3.9: FTIR Spectra of Macrofungus Alumina Composite (MFAI) loaded with (a) Pb (b) Cd (c)Cr

The FTIR spectra is also recorded for Macrofungus based composites loaded with metals. There is no marked variation in peak assignments before and after metal loading. However, a significant augmentation in transmittance is recorded for metal loaded composites as shown in Figure 3.7-3.12. This predicts the surface bonding is enhanced due to incorporation of metal into the composites that are likely to perform better adsorbents.

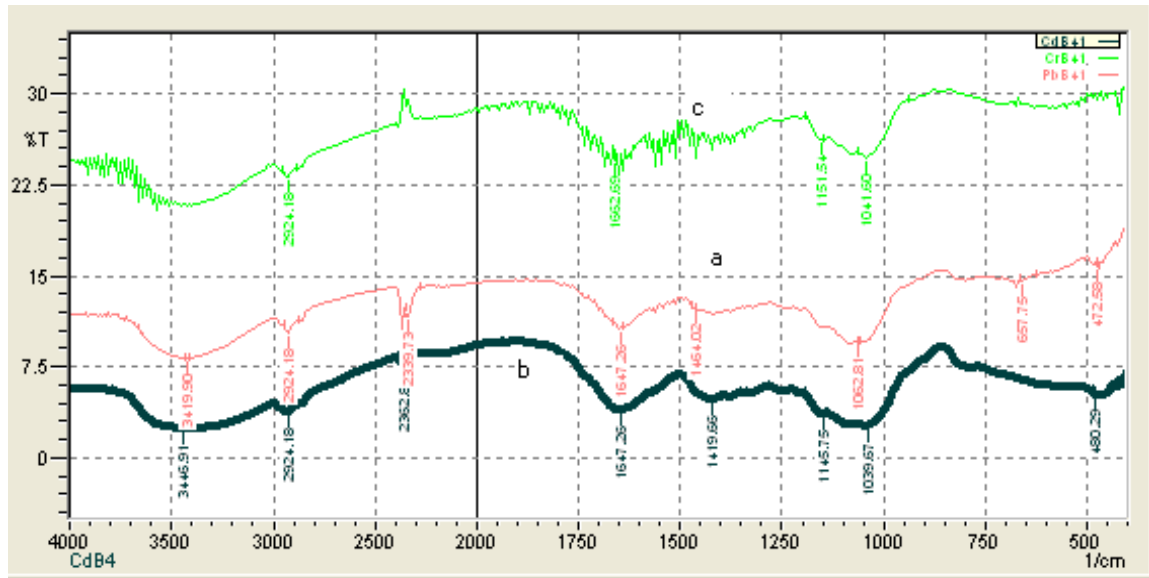


Figure 3.10 FTIR Spectra of Functionalized Macrofungus Silica (FMFSi) loaded with (a) Pb (b) Cd (c) Cr

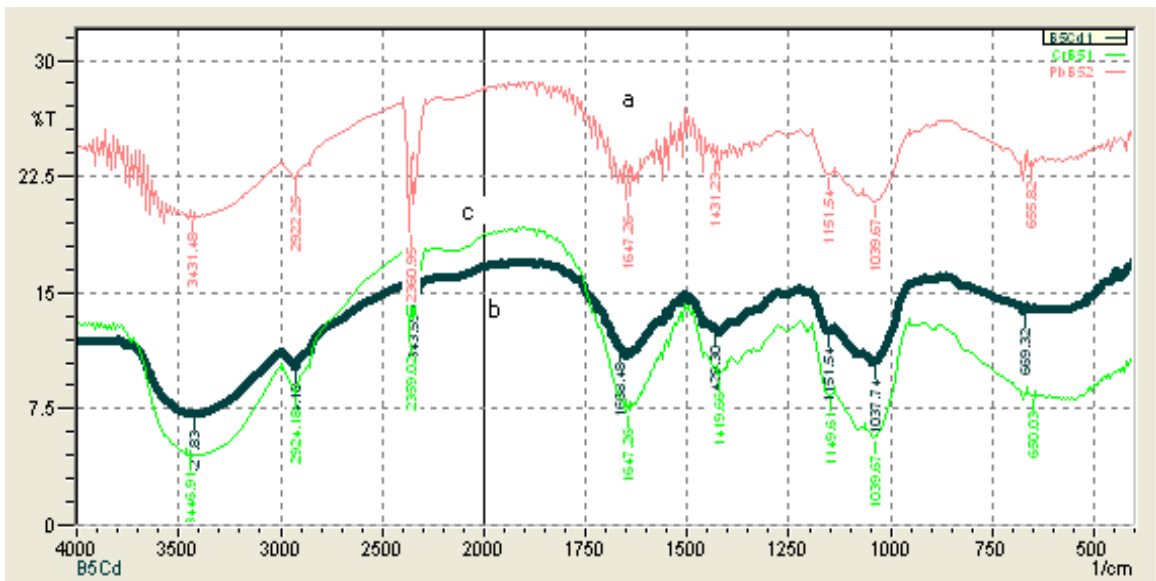


Figure 3.11 FTIR Spectra of Functionalized Macrofungus Alumina (FMFAI) loaded with (a) Pb (b) Cd (c) Cr

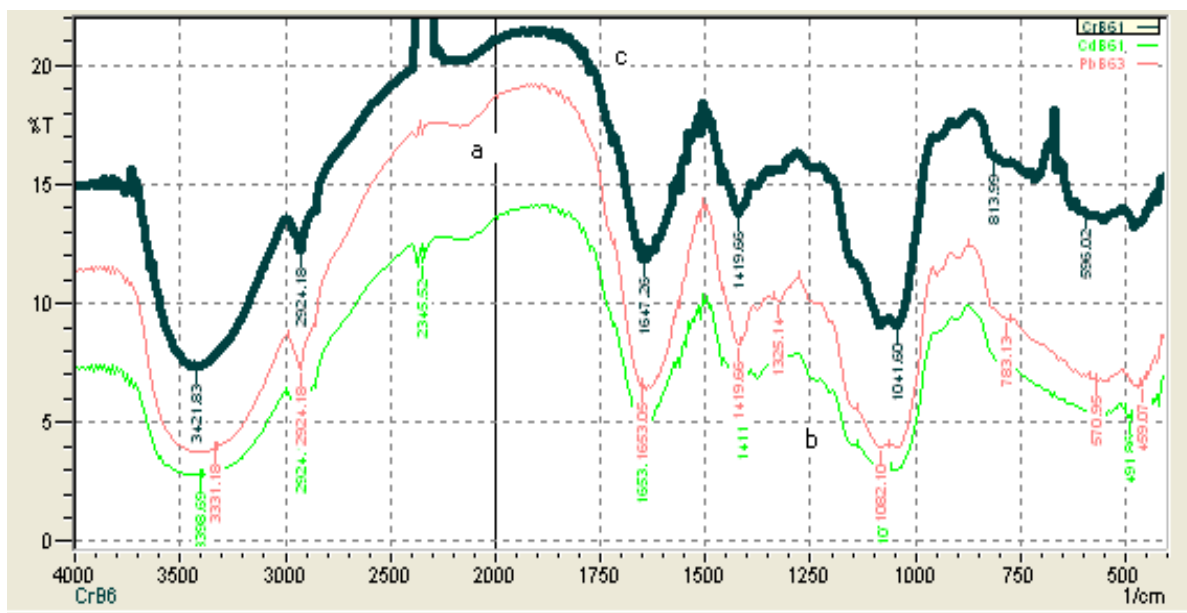


Figure 3.12 FTIR Spectra of Functionalized Macrofungus Si-Al Hybrid (FMFSiAl) loaded with (a) Pb(b) Cd(c)Cr

3.2. Scanning Electron Microscopy

Each of the sample prepared is analyzed for surface characteristics under Scanning Electron Microscope. The Macrofungus (MF) clearly shows the gills of fruiting bodies with interconnected hyphae (see Figure 3.13a). It also suggests that the heterogenous combination of gills and hyphae provides channels and surface porosity for adsorption (discussed later). Its optimal utilization for flow of nutrients and other products is reported by other researchers .

The inorganic precursors (SiO_2 and Al_2O_3) shows finely dispersed particles with crevices and grooves. On comparison, agglomerates of Silica are relatively more pronounced than alumina (see Figure 3.13 b and c). On the other hand, calcium alginate (Figure 3.13d) shows relatively homogenous distribution with packed surface.

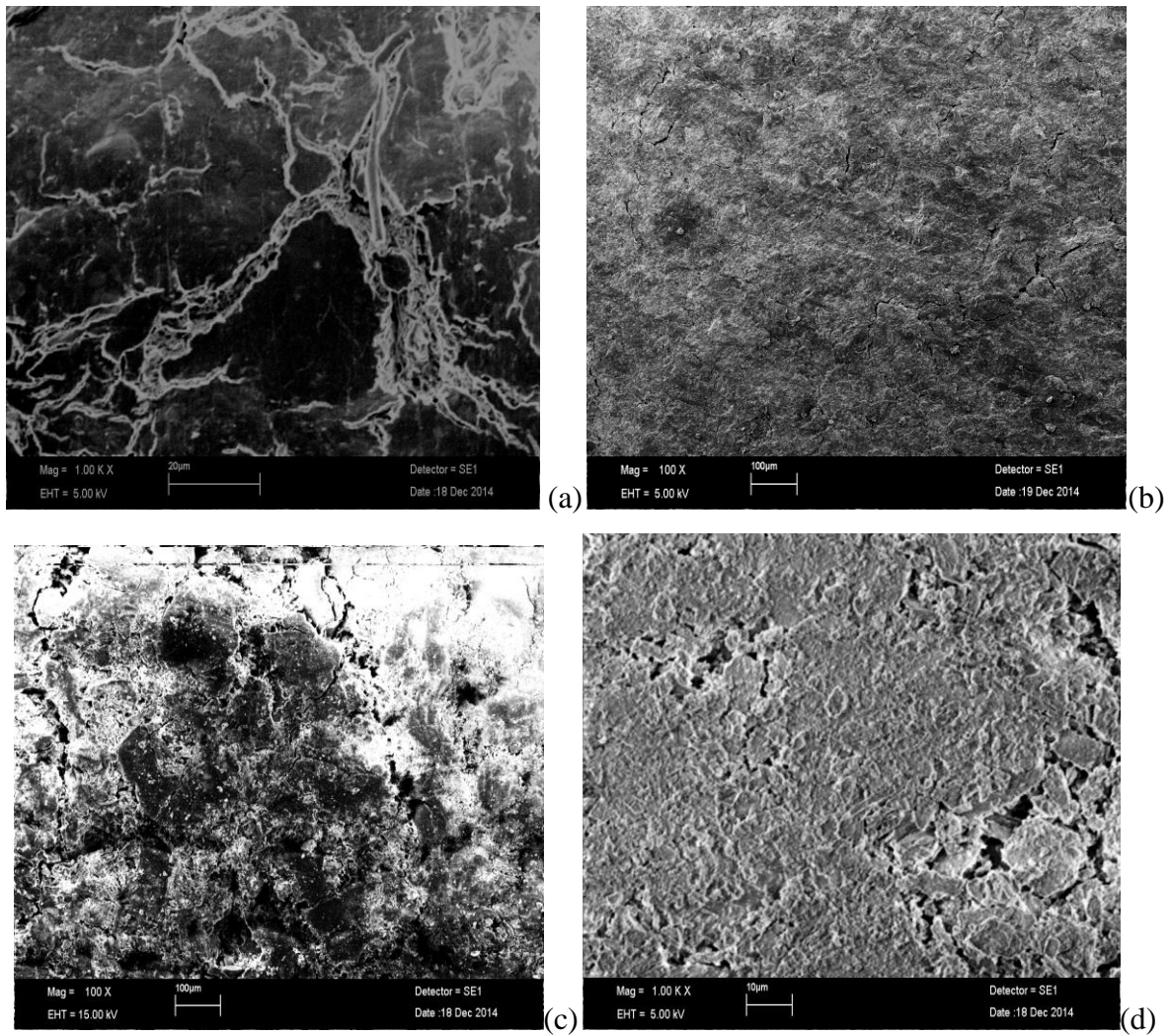


Figure 3.13 SEM Images of (a) Macrofungus (b) Calcium alginate (c) Silica (d) Alumina

Functionalization of Macrofungus with calcium alginate reveals few characteristics features as shown in Figure 3.14(a). The distribution of calcium alginate on the stacks of Macrofungus is clearly evident. It also proposes that linkage is thorough mycelia hyphae, with few particles diffused into the fractures of the Functionalized Macrofungus (FMF).

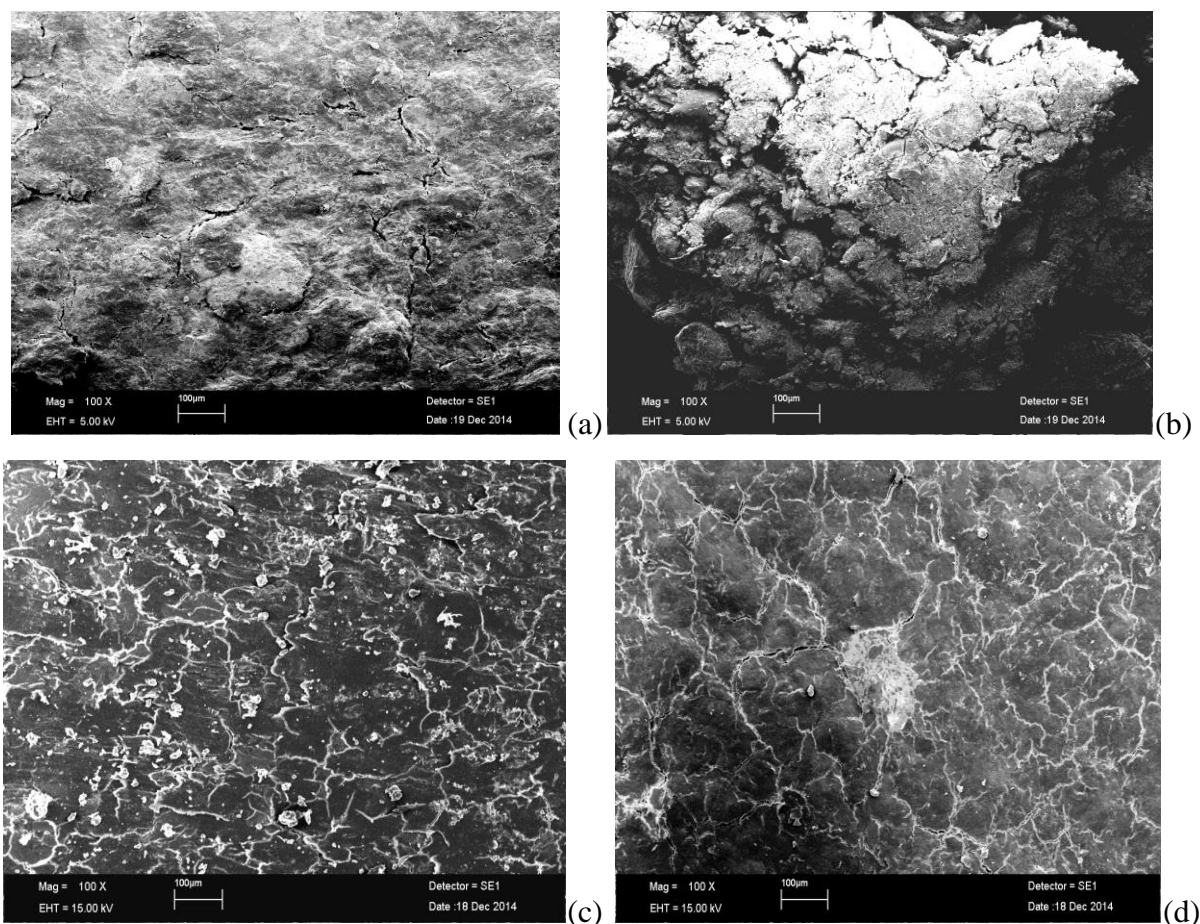


Figure 3.14 SEM Images of (a) FMF (b) FMFSi (c) FMFAI (d) FMFSiAl

SEM of silica and alumina biocomposite with functionalized Macrofungus is shown in Figure 3.14 (b and c). The silica particles are widely distributed on the surface and into the cracks of the structure. An intricate composition develops that proposes the induced silica particles are connected directly through Calcium functionalization and indirectly through hyphae of Macrofungus. On the other hand, alumina particles are beautifully dispersed on the surface of functionalized Macrofungus that provides a base to the alumina. It also suggests that alumina particles are directly linked via Calcium of calcium alginate. The SEM results propose that composite of silica and alumina is good adsorbent for the removal of pollutants (discussed later). However, direct linkage expects chemisorption to be the dominant mechanism for the later composite and both direct and indirect connection for the earlier provides adsorption to take place through physisorption and chemisorption.

The hybrid of functionalized Macrofungus with silica and alumina in the ratio of 10:1:1 under SEM (see Figure 3.14d) support the former discussion of individual biocomposite. The alumina particles are seen on the surface only, whereas silica is distributed on and into the crevices of the structure. Based on these characteristics features, it is expected that hybrid will contribute in adsorption through physical and chemical bonding.

Pursuing the objective of the present study to apply the biocomposites as adsorbents for the removal of selected metals, SEM of loaded adsorbents under optimum operating conditions is recorded. The Macrofungus (50 mg) induced with 25 mg/L of Pb, Cd, and Cr is scanned and images are shown in Figure 3.15(a-c).

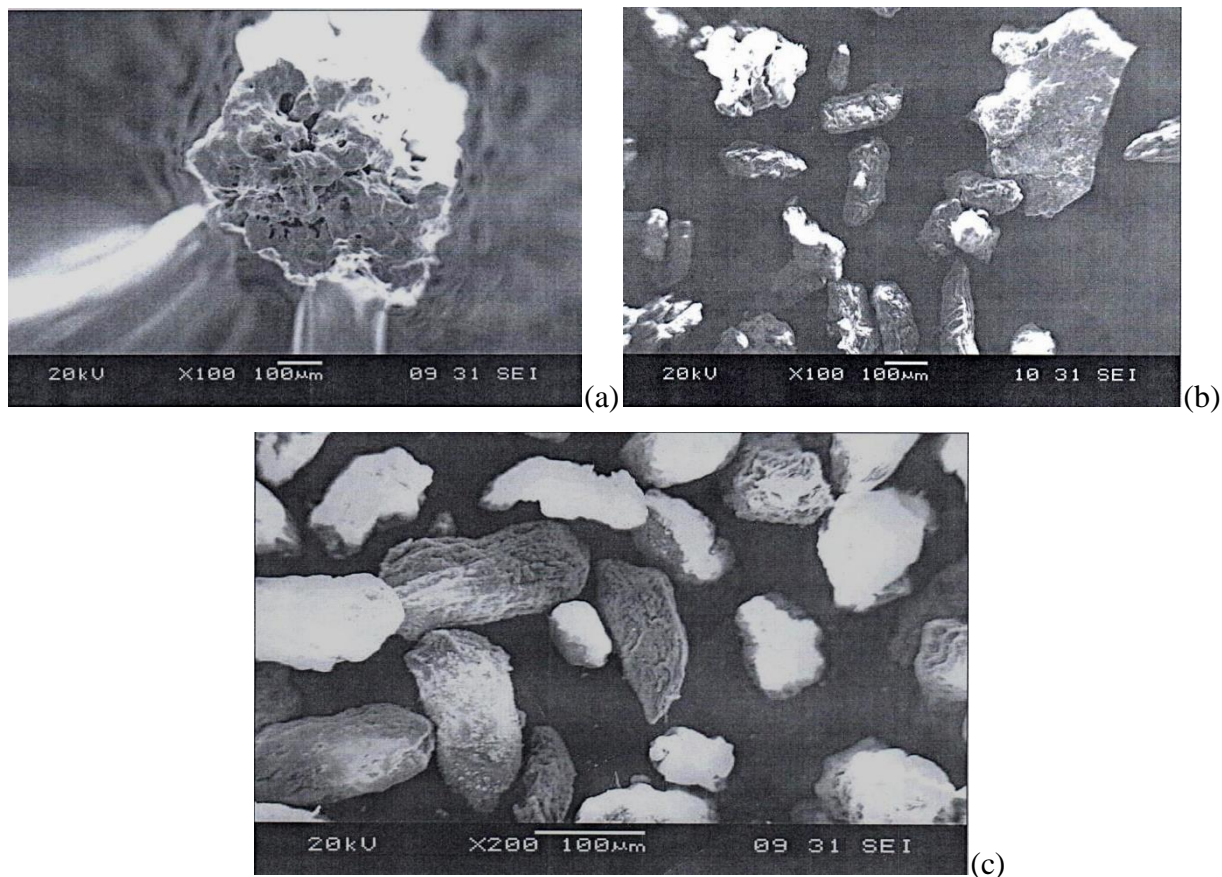


Figure 3.15 SEM Images of Macrofungus loaded with (a) Lead (b) Cadmium (c) Chromium

Loading of the respective metal is generally observed on the surface with intimate contact of the fungal hyphae. The results reveal successful impregnation of each metal. The bunched appearance of lead onto the stalk of Macrofungus is visible in Figure 3.15a. On the other hand, Cadmium and

Chromium is found accumulated in a regular alignment on specific phase of Macrofungus, with more dense population of the Cr Figure 3.15b and c.

An attempt is made to scan the Macrofungus when induced collectively with the three metals (Pb, Cd and Cr). SEM (see Figure 3.16) depicts distribution of metal particles of varying size on the surface of Macrofungus. However, it is difficult to distinguish the specific metal particles. It is proposed that relatively larger particles are of Lead owing to higher molecular weight. The smaller particles nicely scattered belong to Cd and Cr.

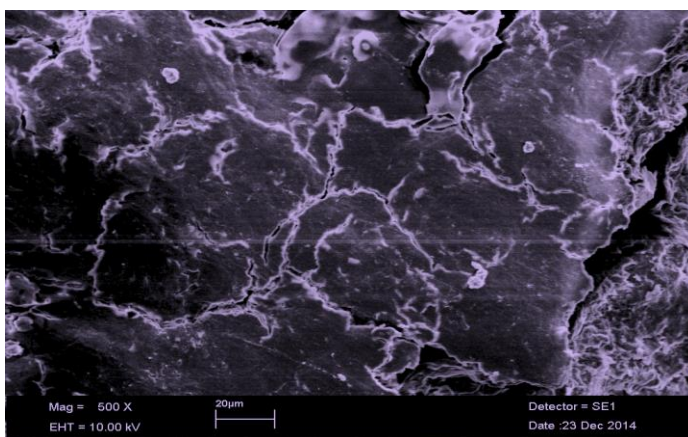


Figure 3.16 SEM Image of Macrofungus loaded with three Metals

Each of the metal is also introduced to Functionalized Macrofungus and subjected to SEM (shown in Figure 3.17 a-c). The introduction of lead shows a wide scattering in comparison to accumulation in a bunch on non-functionalized Macrofungus. This might be attributed to the development of linkage of Lead particles with the Calcium (functionalization of Macrofungus with calcium alginate). FTIR (discussed earlier) also support this variation of functionalized (FMF) and non-functionalized Macrofungus (MF). Similar analysis can be drawn for loading of cadmium and chromium. It is interesting to observe the regular alignment with additional linkage of metal particles with calcium alginate. This proposes that accumulation of metals onto specific sites is suppressed due to its probable involvement in chemical bonding.

The residue of spent silica and alumina based functionalized biocomposites after batch adsorption for removal of individual metals is collected and compressed into pellet for scanning under Electron microscope. The images are shown in Figure 3.18(a - b). It is noted that Pb, Cd and Cr is distributed with indistinguishable features of specific element. Further, silica based composite

reveal more scattered metal distribution in comparison to alumina composite (see Figure 3.18 (a& b)). It also proposes that functionalized Macrofungus to metals ratio is higher in the later. It can be concluded that lower metal content is loaded in the alumina than silica biocomposites.

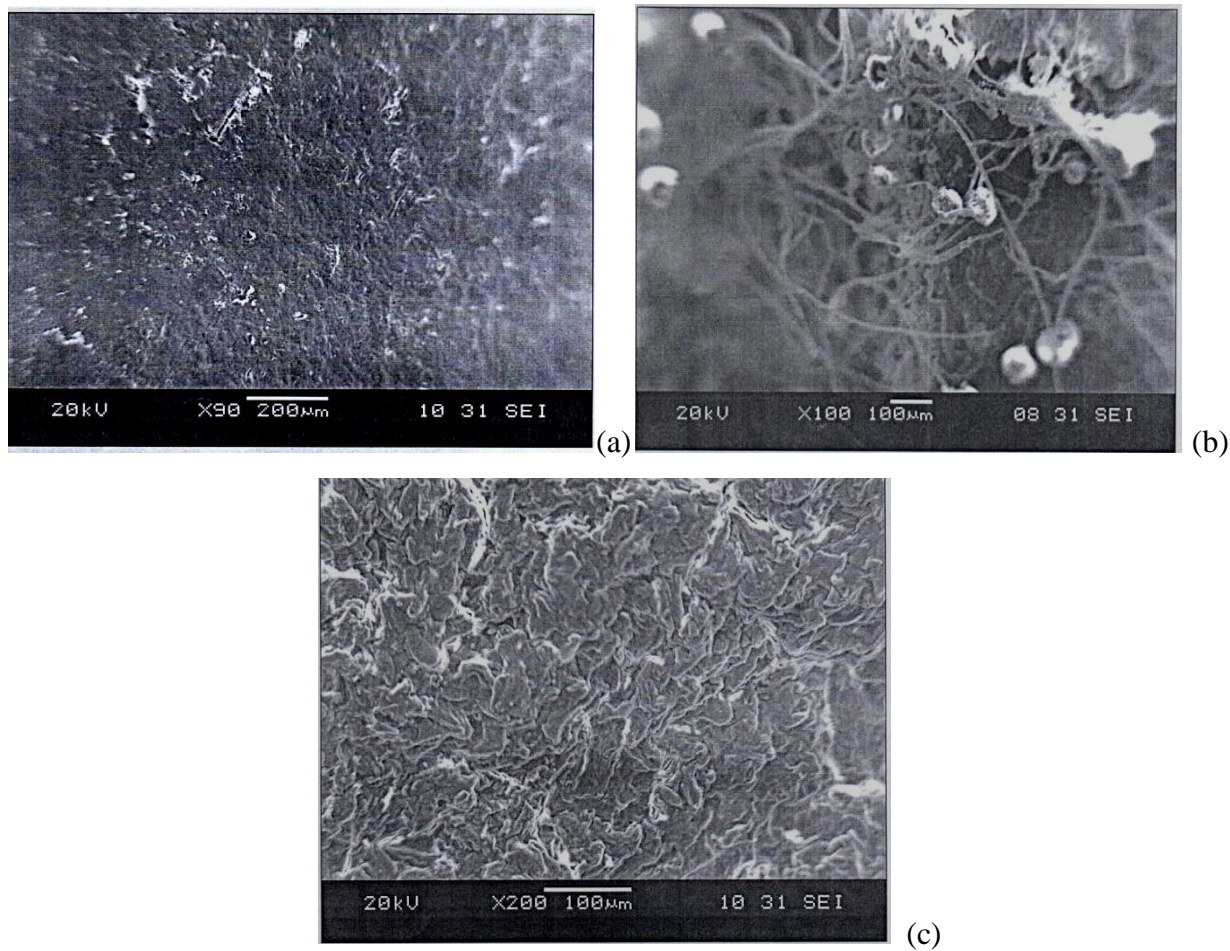


Figure 3.17: SEM images of FMF loaded with (a) Lead (b) Cadmium (c) Chromium

The silica-alumina hybrid biocomposite loaded with three selected metals (see Figure 3.18 c) reveal heterogeneity as major surface characteristic. The peculiar hyphae of functionalized Macrofungus, connected with inorganic moieties of silica and alumina and scarcely distributed metal particles giving a beautiful combination of a hybrid is visible in the micrograph.

It is important to mention that an attempt of preparing composites of Macrofungus with inorganic moieties (SiO_2 and Al_2O_3) is not reported in the literature. The present research enjoys novelty in this regard.

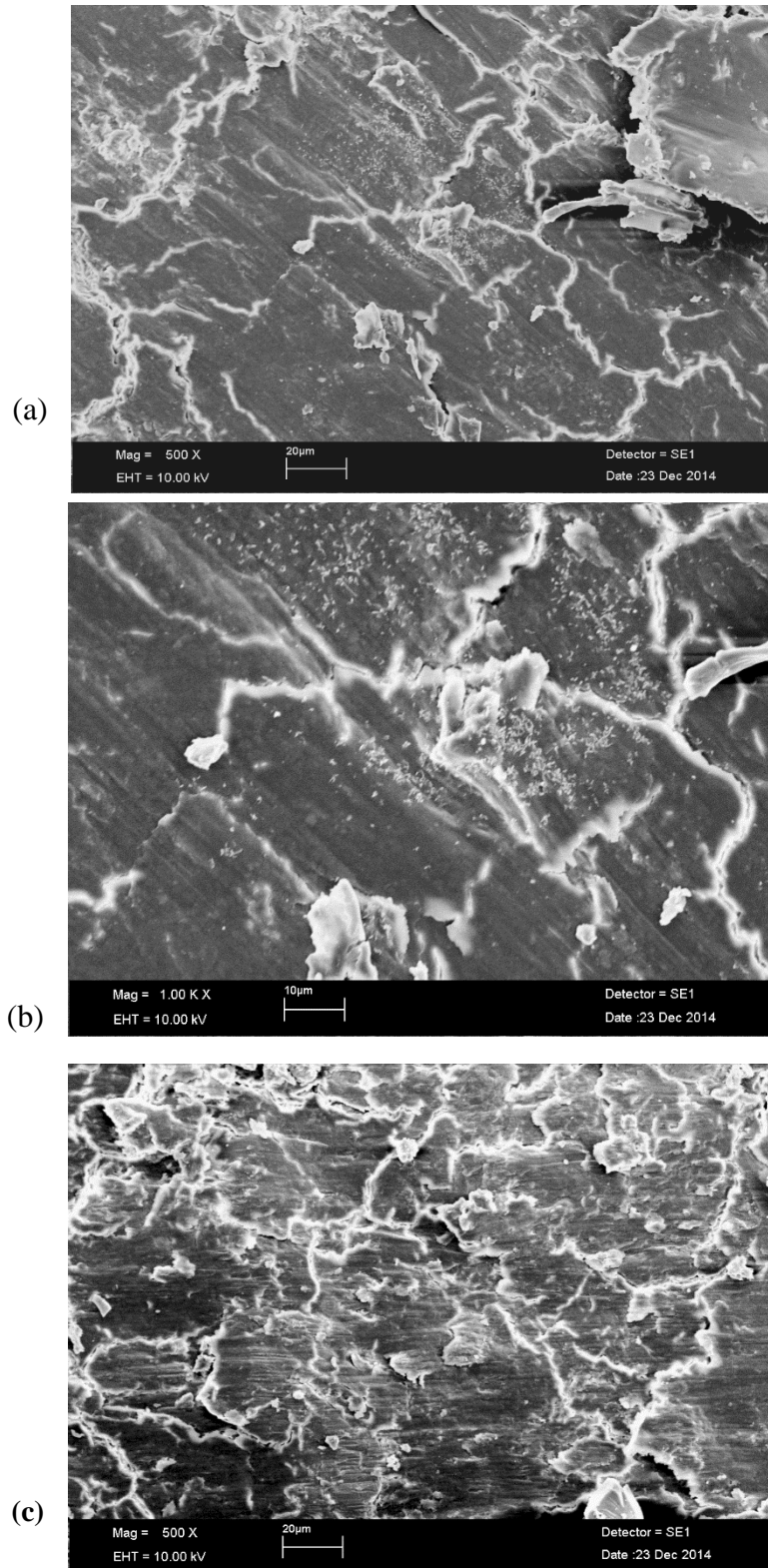


Figure 3.18 SEM images of Metal loaded biocomposites (a) FMF Si (b) FMFAI (c) FMFSiAl

3.3.EDX Analysis

Electron Dispersive X-Ray Spectroscopy is an important tool to determine the elemental composition of the material. This technique is employed on the biocomposite prepared. The results are summarized in Table 3.1. The Macrofungus (MF) is high in protein content. This is indicated by significantly high percentage of Nitrogen element in EDX results. The nitrogen content is omitted and not included in the results as its inclusion in the results minimizes the relative percentage of other elements (adsorbed metals) to a negative value. The Carbon and Oxygen is found as major and Phosphorus as minor element. The inorganic precursors of Al_2O_3 and SiO_2 identify 49% and 39% of Al and Si, respectively. It is interesting to note that Oxygen content is more in both materials owing to ratio of oxygen to element i.e. $2/3$ and $1/2$.

The functionalized Macrofungus (FMF) shows 4.82% of calcium attributed to introduction of calcium alginate $(\text{C}_{12}\text{H}_{14}\text{CaO}_{12})_n$. This confirms the successful functionalization. It can generally be concluded that Carbon and Oxygen are the essential ingredients of each material. This is further supported by the FTIR results (discussed earlier) identifying assignment of functional groups with $-\text{CO}$ linkage. The silica and alumina composite with Macrofungus (non-functionalized) impart significant contribution of the inorganic moiety in the successful preparation of composite. The EDX results shown in Table 3.1 indicate 13% and 22% Si and Al, in respective order.

Furthermore, silica and alumina biocomposite of Functionalized Macrofungus also reveal encouraging results. The relative decrease in calcium content and increase in Oxygen percentage in comparison to functionalized Macrofungus (FMF) is a signature of binding of Silica with calcium alginate in the composite (FMFSi). Similarly, alumina biocomposite (FMFAl) fulfills the requirement to the percentage of 25% aluminum (see Table 3.1). Interesting comparison can be drawn for two biocomposites. Significant higher Al content than Si suggests strong bonding of functionalized Macrofungus with the former. It is expected that this bond strength will offer resistance to the diffusion of incoming pollutants (discussed later in Adsorption application). Further, acidic pH of the base material (FMF) develops strong association to incoming alkaline species (Al_2O_3). The EDX results are duly demonstrated in the FTIR results where $-\text{SiO}$ and $-\text{AlO}$ bonding is indicated in the silica and alumina biocomposite. An attempt is made to assess the elemental composition of the biocomposites when loaded with the selected metals (Pb, Cd, and Cr). Results are shown in Table 3.1.

Table 3.1:Elemental Composition of Macrofungus based Biocomposites(a) without Metal loading (b) with Metal loading

(a) EDX Analysis of Biocomposites without Metal loading										
<i>Sample Codes</i>	C	O	P	Ca	Pb	Cd	Cr	Si	Al	Na/K/Cl
MF	45.24	31.5	2.77	-	-	-	-	-	-	9.75
FMF	60.11	29.58	3.07	4.82	-	-	-	-	-	-
Al ₂ O ₃	-	61.93	-	-	-	-	-	1.90	49.03	-
SiO ₂		71.43						28.57		
MFSi		81.66						14.87		-/3.5/-
MFAI		77.67							15.24	6.53
FMFSi		72.95		4.43				17.77		4.85
FMFAI		62.32		2.68		1.20	0.48	3.76	25	6.24
FMFSiAl		68.09		8.44	3.27		10.47	14.85		3.10
(b) EDX Analysis of Biocomposites with Metal loading										
MF*	61.63	28.13	-	-		10.24				
	66.13	19.68	0.97				13.23			
	68.62	25.51			5.88					
FMF*	65.50	30.27	3.99	5.14		0.49				-/1.92/-
	43.33	36.21		18.43	1.15			0.87		
	71.78	18.2		0.67			9.36			
MFSi(B ₁)*		63.22		8.47	5.75	0.56	0.53	13.43		15.80
MFAI(B ₂)*										
FMFSi(B ₃)*	29.36	48.74		4.33	5.53	0.31	1.56	14.4	3.42	1.71
FMFAI(B ₄)*	62.32		2.68		1.20	0.48	3.76	25	6.24	
FMFSiAl (B ₅)*	68.09	-	8.44	3.27		10.47	14.85		3.10	

The loading of each metal on the Macrofungus is found to follow the sequence $\text{Cr} > \text{Cd} > \text{Pb}$. The minimum incorporation of Lead might be attributed to its higher molecular weight. Similarly, metal loading on functionalized Macrofungus clearly demonstrates the incorporation of each element in the weight percentage of 1.15 (Pb), 0.49 (Cd) and 9.36 (Cr). The optimum loading of Cr suggests that divalent calcium (calcium alginate) offers better coordination to higher valent state elements (Cr^{+3} , Cr^{+6}) in comparison to (Pb^{+2} and Cd^{+2}).

The composite of silica and alumina with Macrofungus (non-functionalized and functionalized) is induced with known concentration of metal salt solution. The MFSi and MFAI samples identify the elemental composition of Pb, Cd and Cr as 5.75%, 0.56 %, 0.53% and 6.68%, 0.23%, 0.60%, respectively. On the other hand, functionalized composites are noted to have decrease loaded metal percentage with comparable Si and Al content. This is rationalized on the basis that functionalization with calcium alginate diffuses into the bulk matrix, thus reducing the available space for metal loading in comparison to non-functionalized Macrofungus.

The functionalized Macrofungus Si-Al hybrid is also assessed for the uptake of metals into the framework. It is interesting to note the appreciable content of each metal with significant amount of silicon and aluminum justifying the hybrid formation.

The EDX results conclude the successful loading of the respective metal, inorganic moieties (SiO_2 and Al_2O_3), calcium alginate (for functionalization), and combination of two and more components in the biocomposites.

3.4.BET Analysis

The surface area (m^2/g) is determined by BET measurements for the Analytical Reagent Grade Silica, Alumina, and Calcium-alginate used for the preparation of composites with Macrofungus. The results show that surface area of SiO_2 , Al_2O_3 and $(\text{C}_{12}\text{H}_{14}\text{CaO}_{12})_n$ is $315\text{m}^2/\text{g}$, $120\text{m}^2/\text{g}$, and $1.62\text{m}^2/\text{g}$, respectively.

It is clearly evident that the inorganic moieties have significantly higher surface area in comparison to organic polymeric specie of calcium alginate. The reduced surface area of the polymer is likely the outcome of polymerization depicting linkage of 'n' number of monomeric units. It also

suggests that more cross-linking into the inner coordination sphere squeeze the external surface area.

It is also observed that the expanded surface area of pristine alumina is downsized on preparation of its composite with the Macrofungus. The composite (MFAl) revealed a surface area of 2.93 m²/g. This demonstrates the successful synthesis of the composite and major participation of the organic moiety (Macrofungus). This is in agreement with the synthesis ratio of Macrofungus: Alumina (10 :1) in the composite (MFAl) (see the Experimental section).

3.5.Determination of Active Sites

Boehm titration method is used to determine the active sites on the surface of the prepared biocomposites. The objective is to estimate the efficacy of these biocomposites as adsorbents for the removal of toxic metals. It is also possible to determine the functional groups available on the surface for linkage. The amount of surface groups is calculated by difference in amount of surface functionality reacted for acids and bases. The results are summarized in Table 3.2.

It is understood that NaOH reacts with all surface groups (carboxylic acids/ carboxyl, lactones, and phenols), NaHCO₃ only with carboxylic groups, Na₂CO₃ reacts with carboxyl and lactones and the difference between NaOH and Na₂CO₃ denotes the number of phenols on the surface.

It is noted that active sites of Macrofungus is overwhelmingly occupied by Carboxylic (-RCOO) functional groups on its surface. On the other hand, the negligible amount of alcohols and lactones is identified by negative value. It can be concluded that the net surface of Macrofungus is basic in nature (total basic value is 2.25). This confirms the protein composition of Macrofungus. On the contrary, this basicity is reduced in functionalized Macrofungus (see Table 3.2). This is expected that adsorption will be lower when calcium alginate is induced on the Macrofungus.

The composites of Macrofungus with silica and alumina depict that amount of active sites is attributed to lactones and carboxylic groups. The two composites can be differentiated as only MFAl being basic. On the other extreme, functionalized composites of silica (FMFSi) and alumina (FMFAI) are enjoying the total acidity. A significant amount of lactones are determined for FMFSi. This suggests that removal efficiency of this composite is contributed through binding of metal with ketones (lactones).

The determination of active sites concludes that development of biocomposites as adsorbents is facilitated through functional moieties of carboxylic, followed by lactones and phenols.

Table 3.2: Determination of Active Sites (Meq/g) of Macrofungus Biocomposites

<i>Functional Groups ▶ Sample Code ▼</i>	<i>-ROH</i>	<i>-RC=O</i>	<i>-RCOO</i>	<i>Total acidic</i>	<i>Total basic</i>
<i>MF</i>	-4.12	-2.925	2.375	-4.675	2.25
<i>FMF</i>	-3.70	-1.785	0.75	-4.735	-1.4
<i>MFSi</i>	-3.17	1.90	0.825	-0.45	-0.80
<i>MFAI</i>	-3.00	2.20	0.45	-0.35	2.25
<i>FMFSi</i>	-2.73	4.45	-0.25	1.465	-1.225
<i>FMFAI</i>	0.97	0.50	-1.325	1.5	-0.9
<i>FMFSiAI</i>	-2.17	-0.85	0.625	-2.4	-1.4

CHAPTER 4 - APPLICATION OF FUNGAL BIOCOMPOSITES FOR REMOVAL OF METALS

Each of the prepared biocomposite is designed as an adsorbent in a batch protocol for the removal of the selected metals as a function of time.

4.1. Effect of Contact Time

The batch experiment is designed to determine the effect of time. It is generally observed that time is an independent parameter for adsorption. The biocomposites (non-functionalized) including Macrofungus (MF) alone, Macrofungus with inorganic moieties of Silica (MFSi) and Alumina (MFAI) applied as adsorbents for the removal of Lead. Results are graphically presented in Figure 4.1(a& b). It is interesting to note that Macrofungus depicts a randomized pattern of adsorption-desorption process for the initial contact time (upto 27 minutes) followed by a relatively increasing trend of adsorption upto maximum of 99 %. This is proposed that an instantaneous equilibrium is attained by the fungal biomass in response to change in contact time. On the other hand, silica and alumina based composites are exhibiting more uniform trend in adsorption. This suggests that the inorganic moieties have a stabilizing effect on Macrofungus thus resulting in reduced adsorption of Lead. The contribution of Alumina is relatively lower in the removal of lead than Silica. This may be related to relatively higher molecular weight of Al_2O_3 providing resistance to incoming particles.

The Functionalized Macrofungus (FMF), its composites with silica (FMFSi), alumina (FMFAI), and hybrid (FMFSiAl), also demonstrated encouraging results for the removal of Lead. The percentage removal varies from 88% to 98% with increasing time. An incremental increase in adsorption with increasing time is noted for the adsorbent with inorganic moieties. However, an increase followed by decrease is observed for functionalized Macrofungus. It appears that functionalization with calcium alginate adds relatively stabilizing effect in comparison to non-functionalized Macrofungus. The sudden and abrupt jumps with kinks in adsorption are subdued due to incorporation of calcium.

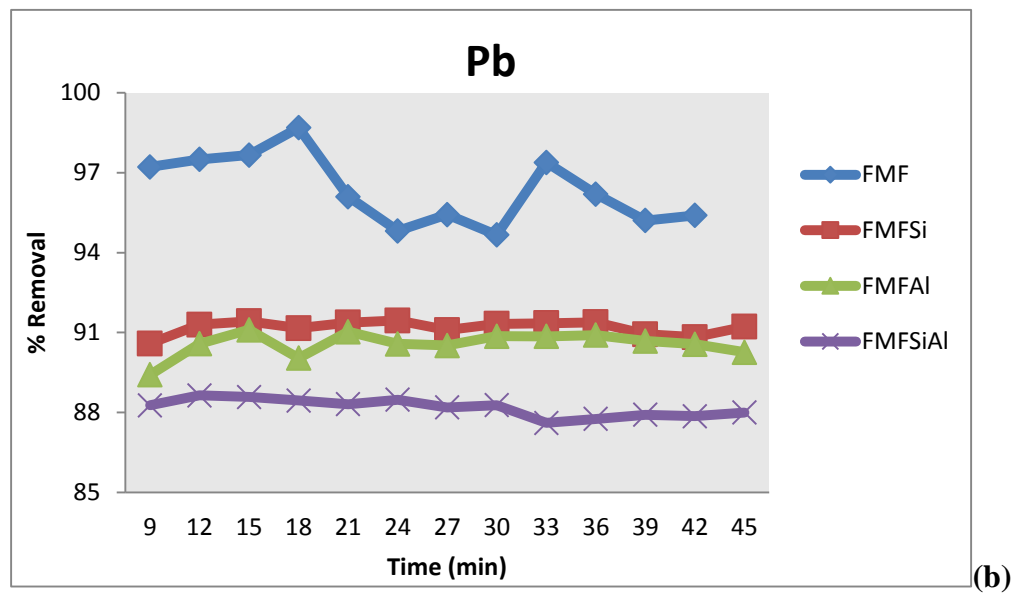
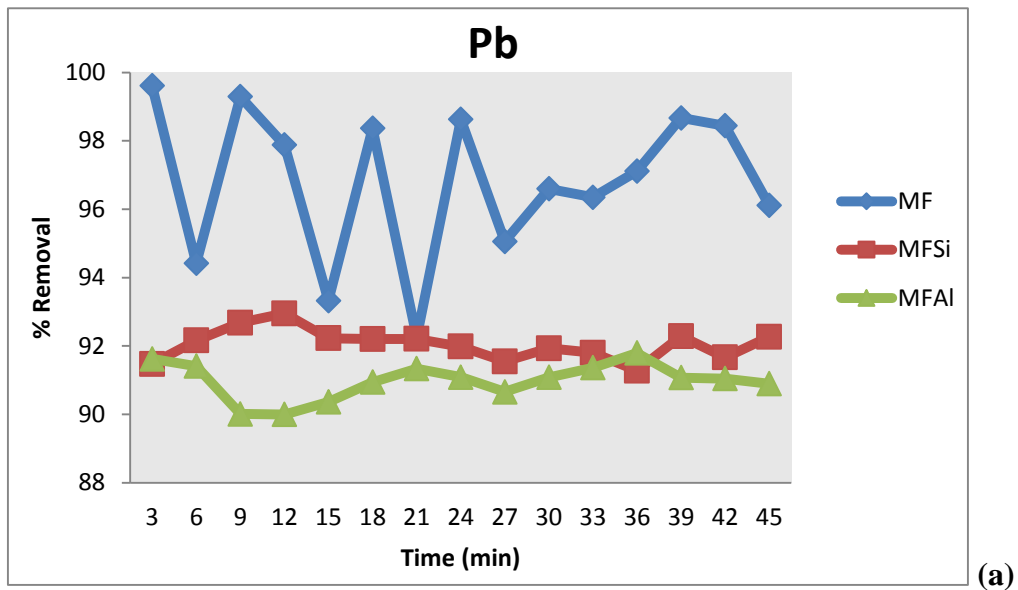


Figure 4.1: Removal of Lead by (a) Non-functionalized Fungus Biocomposites (b) Functionalized Fungus Biocomposites

The biocomposites prepared are also applied as adsorbents for the removal of Cadmium. The results of batch adsorption are shown in Figure 4.2(a & b). It can be seen that efficiency of each adsorbent is comparable and respond almost similarly to the function of time. The adsorption trend is not direct and lead to instantaneous attainment of equilibrium even at time interval of 3 minutes. However, the adsorbents are found to follow the general sequence of MF > MFSi > MFAl, as noted earlier for removal of Lead.

Contrary to this, the functionalized biocomposite depicts less adsorption of Cd on functionalized Macrofungus and Al-based composite to be the effective adsorbent (see Figure 4.2b).

It can generally be concluded that inorganic moieties have a stabilizing and destabilizing effect for the removal of Lead and Cadmium ions from the solution, respectively. It may be postulated that inorganic constituents contribute less effectively in the adsorption of metal ions with higher molecular weight (Pb: 207 > Cd: 112). Further, it is also understood that functionalized Silica and alumina become better adsorbent due to its binding with calcium alginate.

An attempt to remove Chromium from the aqueous solution through adsorption on the surface of Macrofungus biomass, its biocomposites with silica and alumina depicts a kink at varying time of 30 minutes, 42 minutes, and 24 minutes, respectively. This proposes that a physisorption well develops as a result of contact between the adsorbate and adsorbent. A relatively deeper well act as a barrier in case of Macrofungus suggesting more energy is required for the adsorption process, thus less removal of Chromium is attained in comparison to -Si and -Al composites (see Figure 4.3a).

On the contrary, the -Si and -Al composites of Functionalized Macrofungus become more stable showing relatively less adsorption (see Figure 4.3b). It might be attributed to fixing of the biomass due to calcium alginate. The general sequence is FMF > FMFSiAl > FMFSi > FMFAl.

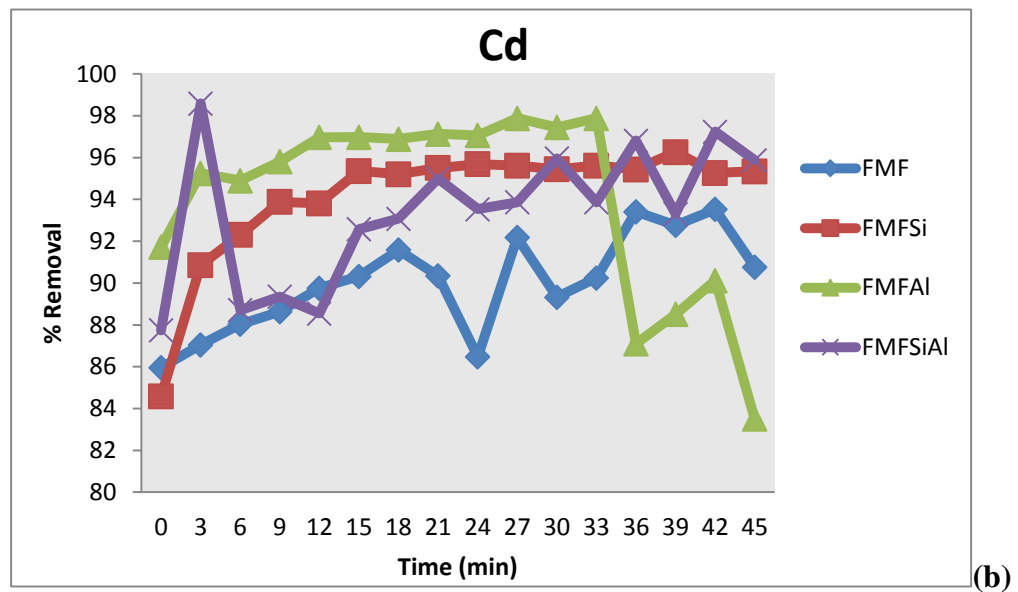
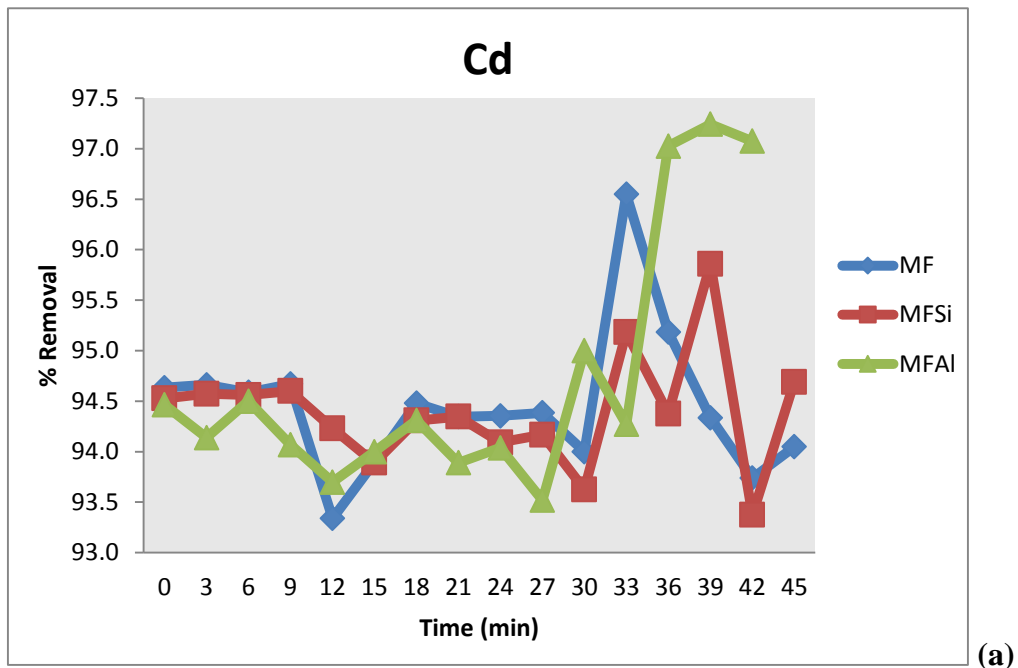


Figure 4.2: Removal of Cadmium by (a) Non-functionalized Fungus Biocomposites (b) Functionalized Fungus Biocomposites

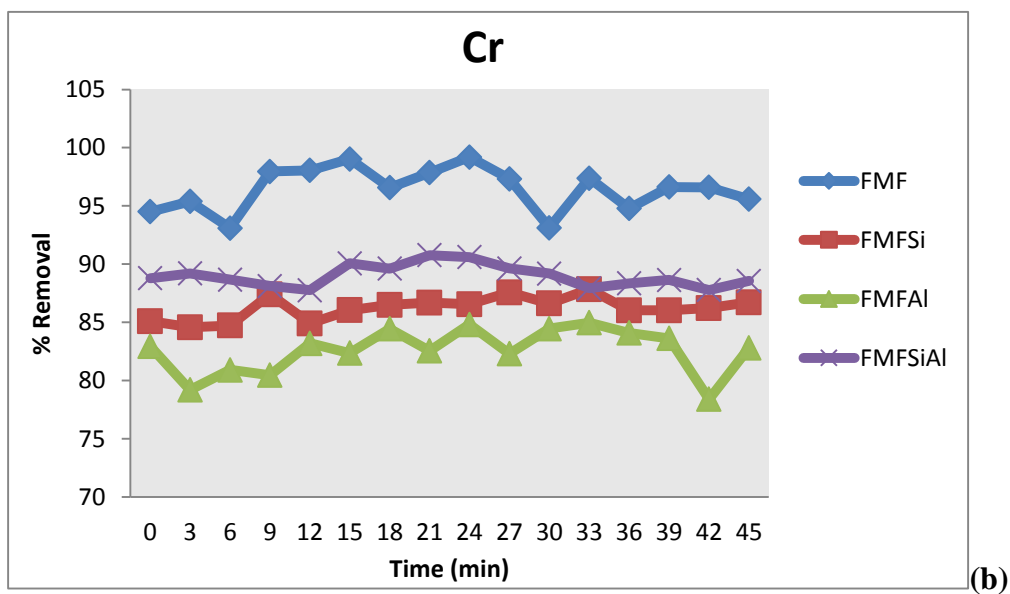
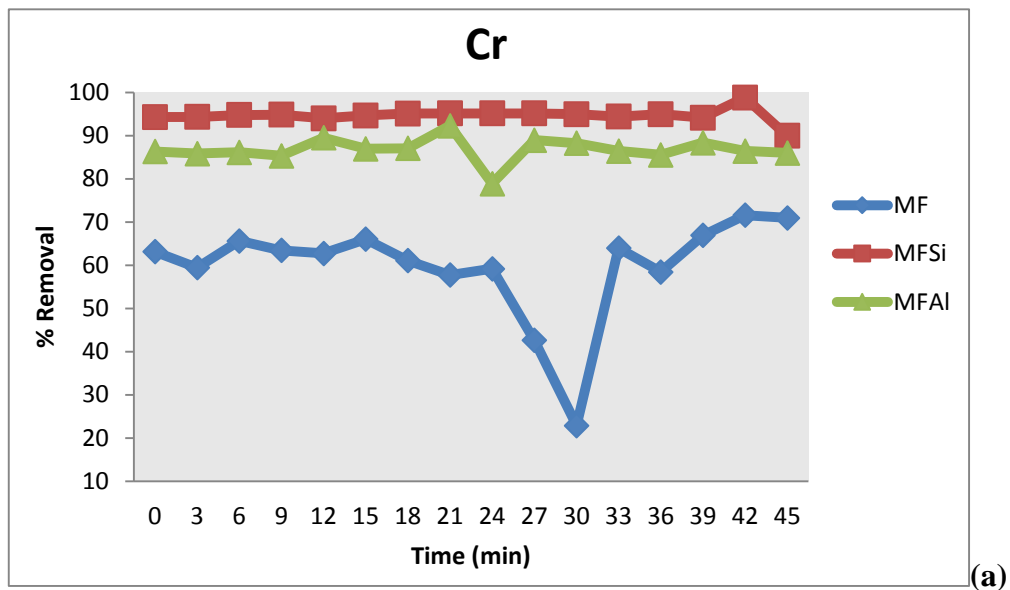


Figure 4.3: Removal of Chromium by (a) Non-functionalized Fungus Biocomposites (b) Functionalized Fungus Biocomposites

4.2. Optimization of pH

The pH is one of the important parameters controlling the removal of pollutants from waste water (Malkoç and Nuhoglu, 2003). Considering this fact, optimization of pH for the batch adsorption experiment on Macrofungus is studied for the removal of Lead, Cadmium and Chromium. The experiment was conducted at wide range of varying pH values covering strongly acidic up to neutral pH. The working metal salt solutions are maintained at pH values of 2, 3, 3.5, 4, 4.5, 5, 5.5, 6 and 7. It is important to mention that alkaline pH was also attempted but due to precipitation and agglomeration of fungal biomass, it was dropped from the study design. Actual pH value of each sample (as control) is also noted for comparison. Other parameters like adsorbent dose and induced metal salt solution concentration is kept constant throughout the experiment.

It is clear from the Figure 4.4 (a-c) that pH has a momentous role on metals uptake. Each working pH is noted to have a significant removal percentage ranging from 91.87% to 96.11% at maximum contact of 45 minutes. The control pH (5.23) removes 71.93% of Lead through adsorption on Macrofungus biomass. It is interesting to note that comparable removal efficiency of 71.06% is depicted at pH value of 5.5. It is concluded that 3.5 pH is the optimum value for the removal of Lead showing maximum adsorption. The removal of Cadmium on Macrofungus at varying pH from control to pH 7 is attempted. It is clearly evident that optimal removal is attained at neutral pH showing a value of 97.18%. On the contrary, control pH is found to have comparable efficiency to pH 5 (see Figure 4.4 b). This might be attributed to the fact that the recorded pH for control sample is 4.8. It may be concluded that a range of strongly acidic (pH 2 to 4.5) and neutral pH can conveniently be applied for the appreciable working of removal of lead. This gives a broad pH window for Cd in contrast to Lead (as noted earlier).

Removal of Chromium is investigated at varying pH to determine the optimum range. Results are graphically presented in Figure 4.4c. It is noted that control (5.64) and pH 5 give comparable results with removal percentage of 81%. However, on close investigation, it is found that the optimum removal of 95% is attained at working pH of 4.5. It can generally be concluded that acidic pH ranges are more suitable for the optimum removal of metal ions from the polluted environment. Following this, more adsorption experiments are conducted at fixed parameters of pH 3.5, adsorbent dose of 50mg, and adsorbate concentration of 25 mg/L.

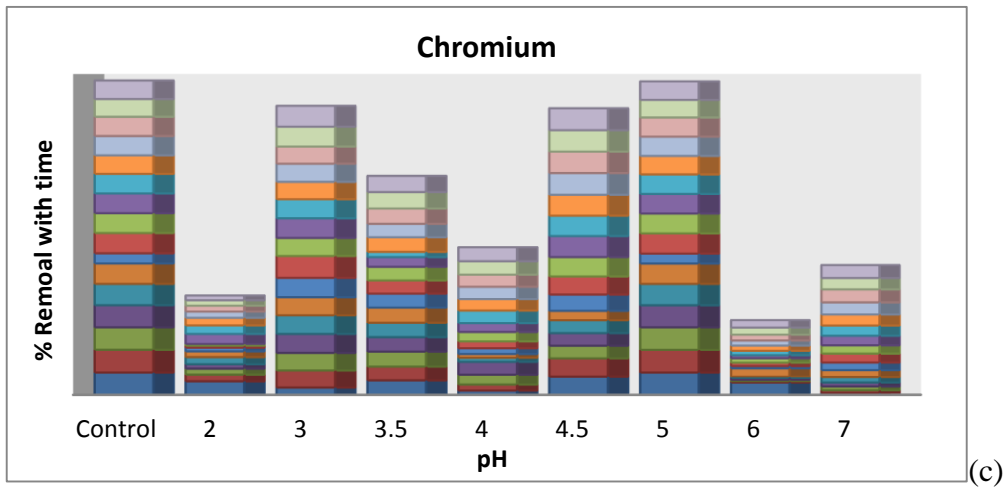
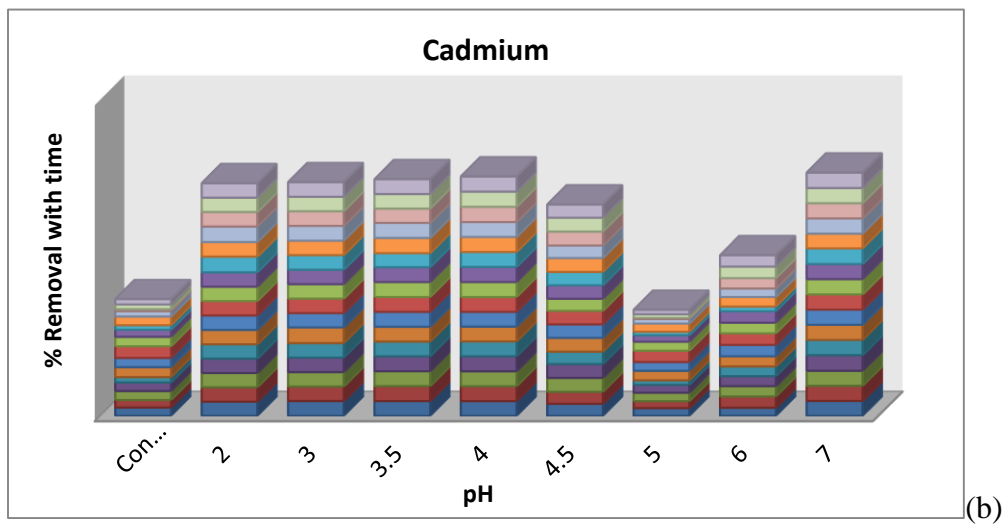
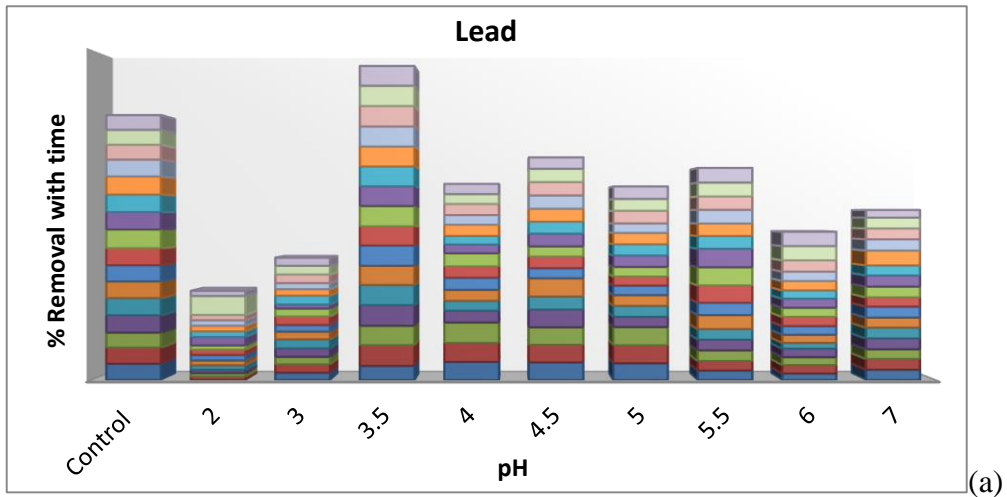


Figure 4.4: Adsorption on Macrofungus at varying pH for Removal of Metals(a) Lead

(b) Cadmium (c) Chromium

4.3. Evaluation of Adsorbents Efficiency

After successful development of biocomposites, each one is applied as adsorbent for the removal of Lead, Cadmium and Chromium under optimum working conditions (defined earlier). It can be seen that efficiency of each adsorbent is appreciable ranging from 89% to 99%. On further probe, it also appears that each non-functionalized composite can conveniently be compared with its functionalized counterpart. For instance, (non-functionalized) MF, MFSi, MFAI and (functionalized) FMF, FMFSi, FMFAI have comparable efficiency to its respective component (see Figure 4.5a).

On average, functionalized adsorbents show decline in adsorption efficiency. It might be attributed to the fixing of biomass on addition of calcium alginate. Further, it is postulated that calcium alginate occupy the space by filling the pores, thus limiting the adsorption efficiency.

This argument is in direct relation to the BET surface area. The significantly higher surface area of Analytical Reagent Grade SiO₂ (315 m²/g) and Al₂O₃ (120 m²/g) in proportion to Calcium alginate (1.62m²/g) justifies more intrusion of the later into the biomass.

Similar attributes are depicted by non-functionalized and functionalized composites with higher efficiency of the former for the removal of Cadmium and Chromium (see Figure 4.5b& c). However, the higher adsorption exhibited by silica-alumina hybrid composite is likely because of the role of inorganic precursors. The literature supports that silica and alumina are good adsorbents for the removal of metals (Afkhamiet al., 2010; Mikuła et al., 2009).

The only discrepancy is noted for functionalized Macrofungus for the removal of Chromium. This biocomposite depicts lower adsorption for Cadmium and Lead in comparison to non-functionalized adsorbents. It is understood that functionalization due to calcium alginate reduces the available pores and thus limits the adsorption of metal ions. On the contrary, more adsorption on functionalized adsorbent (FMF) for Chromium is likely because of the fact that lower molecular weight (Cr) molecules are facilitated in comparison to Lead and Cadmium.

The overall adsorption efficiency of composites followed the sequence:

MF > FMF and MF > MFSi > MFAI > FMF > FMFSi > FMFAI > FMFSiAl

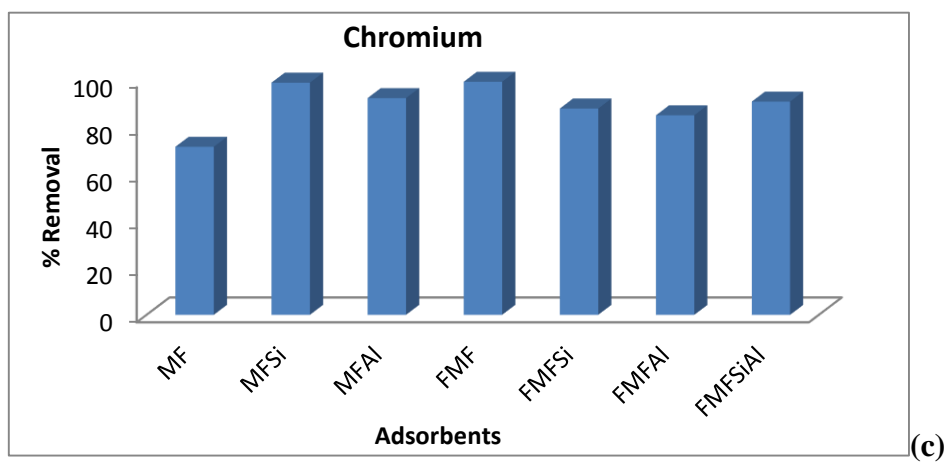
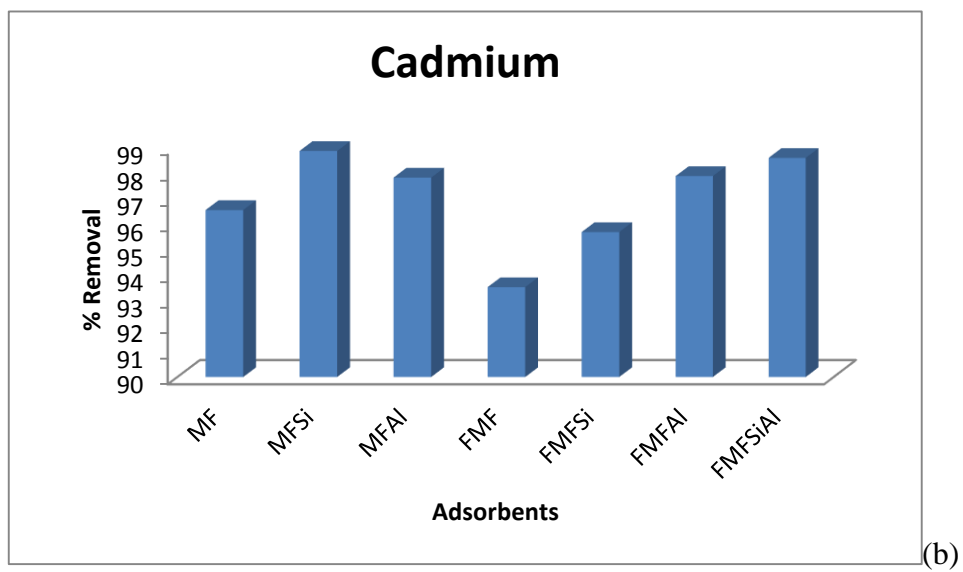
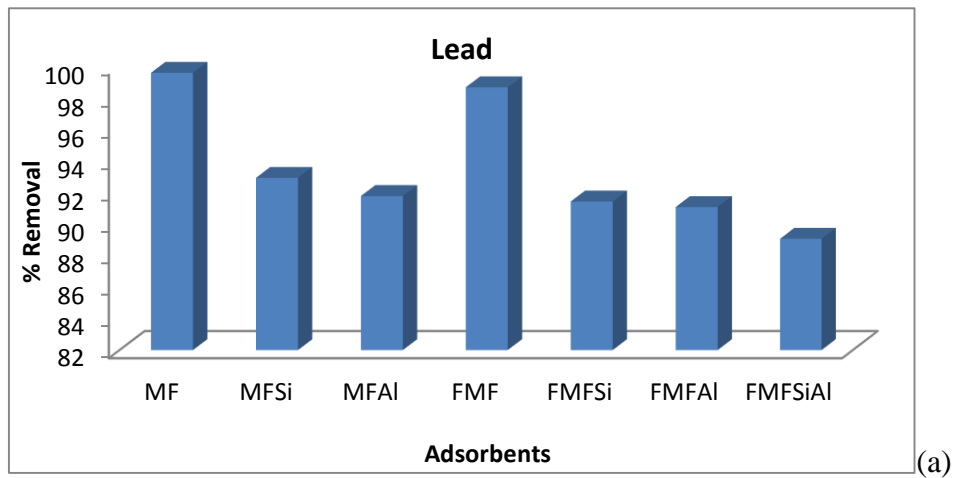


Figure 4.5: Adsorption Efficiency of MacrofungusBiocomposites for Removal of Metals

(a) Lead (b) Cadmium (c) Chromium

4.4. Adsorption Isotherms

Equilibrium isotherms are applied to get an insight of sorption mechanism to propose surface properties and affinity of adsorbents. For practical design of adsorption, isotherms are considered as indispensable data source for adsorbent and adsorbate relationship. A number of equations are applied by researchers for analyzing experimental data. Most frequently experienced isotherms are Langmuir, Freundlich; and Temkin, isotherms. These models are applied onto experimental data extracted from batch sorption studies performed at fixed initial metal ion concentration and sorbent dosage at room temperature for optimum contact time.

4.4.1. Langmuir Isotherm

Langmuir isotherm conventional use is to measure and compare efficiency of different biosorbent (Langmuir, 1916). It was originally developed to signify physisorption of gas on activated carbon, possesses well-defined adsorption sites having the same adsorption energy (Davis et al., 2003). Energetically identical surface binding sites have equal affinity for the sorbate with no interaction among these molecules is main assumption in its formulation (Senthil et al., 2010). So this model is also called the ideal localized monolayer model. For solid–liquid systems, the Langmuir isotherm is given as:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (4.1)$$

Linear form of the Langmuir isotherm is:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad (4.2)$$

where K_L is sorption equilibrium constant (L/mg), q_m is measure of the maximum adsorption capacity (mg/g) in the system; q_e and C_e are sorbed and residual metal ion concentration. A plot of C_e/q_e versus C_e should indicate a straight line of slope $(1/q_m)$ and an intercept of $(1/K_L q_m)$ from which q_m and K_L can be calculated.

4.4.2. Freundlich Isotherm

The Freundlich adsorption isotherm usually fits the experimental data over a wide range of concentrations (Allen and Brown, 1995; Sanchez et al., 1999). Freundlich isotherm gives the

relationship between equilibrium liquid and solid phase capacity based on the multilayer adsorption (heterogeneous surface). The isotherm derivation is based on the assumption that the adsorption sites are distributed exponentially with respect to the heat of adsorption and is given by (Freundlich, 1907):

$$q_e = K_F C_e^n \quad (4.5)$$

Linear form of the Freundlich isotherm is:

$$\log q_e = \log K_F + 1/n \log C_e \quad (4.6)$$

where K_F (mg/g) indicates the multilayer adsorption capacity and $1/n$ an empirical parameter related to the intensity of adsorption. Its value in the range of 2-10 indicates favorable sorption. A plot of $\log q_e$ against $\log C_e$ yields a straight line, the values of constants ($1/n$) and ($\log K_F$) can be obtained from slope and intercept respectively, from which n and K_F can be calculated.

4.4.3. Temkin isotherm

The assumption of Temkin isotherm equation is that the heat of adsorption of all the molecules in layer decreases linearly with coverage due to adsorbent-adsorbate interactions (Temkin & Pyzhev, 1940). The adsorption is characterized by a uniform distribution of the bonding energies, up to some maximum binding energy (Wasewar et al., 2008). The Temkin isotherm is given as:

$$q_e = \frac{RT}{b_T} \ln(K_T C_e) \quad (4.7)$$

On linearization the equation becomes:

$$q_e = B \ln K_T + B \ln C_e \quad (4.8)$$

where $B = RT/b_T$, constant b_T (KJ /mol) is related to the heat of adsorption and K_T (L/g) is the equilibrium binding constant. A plot of q_e versus $\ln C_e$ enables to determine b_T and $\ln K_T$ from the slope and intercept of straight line plot from which B and K_T can be calculated.

Table 4.1:Langmuir, Freundlich, and Temkin Isotherms Analysis against Sorption Variables

Composite →→	MF			MFSi			MFAI			FMF			FMFSi			FMFAI			FMFSIAI			
Metal Pollutant →→	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	
Langmuir Parameters																						
Q _m (mg/g)	95. 23	20. 83	.1 11 1	20. 40	22. 22	18. 51		22. 20	19. 23	16. 94	20. 40	23. 25	17. 85	19. 60	17. 24	19. 19		16. 20	17. 94	21. 36	19. 84	
K _L (L/mg)	52. 5		0. 79	6.1 2		3.3 75		22. 5	1.8 5	3.2 7	4.0 8			6.3 2.8	2.3 7	3.1 2		1.2 10	3.0 29	11. 1		3.6
R ²	0.9 96	0.9 99	0. 93 6	0.9 99	0.9 98	0.9 92	0.9 99	0.9 99	0.9 96	0.9 94	0.9 99	0.9 99	0.9 99	0.9 98	0.9 99	0.9 99	0.9 98	0.9 99	0.9 99	0.9 98	0.9 99	
Freundlich Parameters																						
n	5.0 76	13. 51	8. 26 4	11. 09	27. 70	12. 45	9.8 0	22. 72	6.5 78	8.4 0	9.3 45	33. 78	6.9 4	11. 54	6.2 38	7.8 7	14. 49	4.5 66	5.0 76	13. 51	8.2 64	
K _F (mg/g)	19. 69	7.3 82	7. 43 5	7.3 4	7.3 31	7.6 5	7.2 80	7.5 06	6.4 0	7.2 80	6.9 46	7.1 8	7.1 65	7.4 5	7.2 30	6.9 46	7.6 5	6.0 8	19. 69	7.3 82	7.4 35	
R ²	0.7 38	0.9 6	0. 50 6	0.9 96	0.8 25	0.7 99	0.9 98	0.9 69	0.9 48	0.8 51	0.9 82	0.8 95	0.9 96	0.9 52	0.9 98	0.9 93	0.9 34	0.9 95	0.7 38	0.9 6	0.5 06	
Temkin Parameters																						
B (KJ/mol)	2.3 24	1.6 51	2. 26 4	1.9 77	0.8 45	1.6 97	2.2 29	1.0 05	3.4 02	2.4 91	2.4 18	0.7 13	2.9 69	1.8 82	3.1 65	2.7 64	1.5 15	4.5 4	2.7 27	1.3 21	2.6 76	
KT (L/g)	0.0 02	7.8 5E- 07	3. 75 E- 05	7.6 5E 06	9.0 8E 13	1.8 2E 06	2.6 3E 05	1.2 7E 10	0.0 00 9		3.7 4E 05	2.7 3E 15		0.0 4.9 00 6	0.0 E- 00 6	0.0 00 1		3.6 2E 07		0.0 00 2	1.9 1E 08	9.1 E- 05
R ²	0.8 07	0.9 64	0. 62 2	0.9 96	0.8 4	0.8 33	0.9 98	0.9 72	0.9 6	0.8 73	0.9 86	0.9 01	0.9 96	0.9 59	0.9 98	0.9 95	0.9 43	0.9 97	0.9 98	0.9 02	0.9 98	

The Regression of Coefficient (R^2) close to unity defines the maximum fitness of the experimental data to the Langmuir postulate. The treatment of present study data to Langmuir Isotherm demonstrated R^2 greater than 0.99 for each composite synthesized and applied as adsorbent for the removal of Pb, Cd, and Cr. This confirms the adsorption of metal on the homogenous surface layer of the composites resulting in an intricate adsorbate-adsorbent interaction. The homogeneity of the adsorbent structure is attributed to the ingredient (Macrofungus) commonly present in all the composites synthesized.

This also suggests that monolayer of each adsorbent is completely saturated with the induced metal ion concentration. However, the maximum loading is observed for Lead on the Macrofungus (pristine) demonstrated by optimum q_m (95.23 mg/g).

On the other hand, the decrease in q_m for all the selected metals (Pb, Cd, Cr) on the composites prepared is attributed to the induction of heterogeneity due to other constituents than Macrofungus. This also indicates multilayer adsorption for all the composites of Macrofungus.

On the contrary, the agreement of experimental adsorption data for Freundlich Isotherm is also noted. Close investigation of R^2 values reveal that metals optimally follow the multilayer adsorption on all composites following the sequence, $Cd > Pb > Cr$

This articulates that composites are multilayered structures allowing the uptake of metals on mono sub-layers in accordance with the energy. Further, smaller value of $1/n$ and larger value of K_F indicates that the adsorbent has greater affinity for a particular metal ion over another (Ilkay, 2011). It is interesting to note that negligibly small values of 'n' are calculated for each metal adsorbed on prepared composites (see Table 4.1). On the other hand, relatively higher Freundlich constant (K_F) on Macrofungus (MF) and Si-Al composite of Functionalized Macrofungus (FMFSiAl) indicates optimum preference for Lead adsorption. This preference might be attributed to the higher molecular weight of Lead in comparison to Cadmium and Chromium.

The experimental adsorption data depicts good fitness ($R^2 > 0.96$) generally for all the composites and selected metals to Temkin Isotherm. The only exception is noted for adsorption of Pb, Cr; Cd, Cr; and Pb on MF, MFSi and FMF, respectively. In addition, the negative values of heat of sorption represented as B (KJ/mole) indicate weak sorbate-sorbent interaction proposing a shallow physisorption wells for metals uptake on the surface of composites.

4.5. Kinetic Studies

Kinetic studies suggests that for designing of a good adsorbent the variable parameters, the fitness of pseudo second order (see Table 4.2) indicate dependence of adsorption on more than one factors. In the present study the incorporation of different inorganic materials (Si, Al and both) into the Macrofungus, functionalization/ immobilization with calcium alginate to provide more binding sites. Optimizations of pH for adsorption are few parameters evaluated for development of good adsorbents.

The non-compliance of intra-particle diffusion kinetic also support the present study results that adsorption/ removal of metals is facilitated through binding on the surface functional groups.

4.6. Adsorption Capacity (q_e)

The development of Macrofungus and its composites are applied as adsorbent for the removal of metal ions from the aqueous solution in batch protocol. The experimental data is evaluated for the adsorption capacity (q_e) of each adsorbent and metal removal. The results are summarized in Table 4.2. Based on the adsorption capacity (q_e) value, the biocomposites are classified for adsorption efficiency as good (60-80 mg/g), very good (81-90 mg/g) and excellent (91-100 mg/g).

It is encouraging to note that not a single composite stands below the efficiency of good adsorbent for the uptake of Lead, Cadmium and Chromium. On further probe, it is evident that Macrofungus (MF) shows ‘Good’ adsorption capacity for removal of Lead. On the other hand, FMFSi and FMFSiAl are on the borderline capacity of ‘Good’ and ‘Very Good’ for Chromium and Lead, respectively.

It is interesting to note that Lead is showing ‘Very Good’, whereas Cd and Cr exhibit ‘Excellent’ adsorption on the composites of –Si and –Al with Macrofungus and Ca-alginate Functionalized Macrofungus. The general sequence of adsorbent efficiency of biocomposites for the Metals removal follows:

$$MF < MFSi < MFAl < FMF < FMFSi < FMFAl < FMFSiAl$$

The study of Adsorption Capacity suggests the possible and potential application of each biocomposite as successful adsorbent for remediation of metal pollution.

Table 4.2: Comparison between the estimated adsorption rate constants, q_e , and correlation coefficients associated with the Kinetic Models

Sorbents	MF			MFSi			MFAI			FMF			FMFSi			FMFAI			FMFSIAI		
	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr	Pb	Cd	Cr
qe (exp) (mg/g)	67.0 2	93. 11	98. 46	88. 95	97. 12	91. 72	87. 94	90. 62	96. 04	98. 68	93. 52	99. 19	86. 51	92. 10	80. 56	89. 42	92. 87	86. 02	80. 21	97. 20	90. 62
Zero order Kinetic Parameters																					
K (1/min)	0.14 8	0.0 0	0.0 95	0.0 01	0.0 01	0.0 01	0.0 04	0.0 21	0.0 09	0.1 13	0.0 3	0.0 01	0.0 16	0.0 34	0.0 08	0.0 13	0.0 38	0.0 08	0.0 01	0.0 33	0.0 02
qe (cal) (mg/g)	53.6 7	75. 1	75. 16	66. 98	73. 81	70. 7	66. 27	68. 12	73. 48	78. 34	71. 7	75. 11	65. 46	70. 35	60. 98	67. 6	69. 7	65. 31	60. 33	74. 94	68. 34
R ²	0.49 8	0.0 05	0.1 74	0.0 1	0.0 02	0.0 01	0.1 79	0.6 81	0.0 3	0.4 44	0.5 4	0.0 01	0.2 1	0.4 87	0.2 75	0.3 11	0.2 6	0.0 6	0.0 28	0.3 45	0.0 3
Pseudo First order Kinetic Parameters																					
K ₁ (1/min)	0.00 12	0.0 05	0.0 5	0.0 06	0.0 06	0.0 06	0.0 05	0.0 1	0.0 06	0.0 6	0.0 2	0.0 06	0.0 1	0.0 2	0.0 05	0.0 05	0.0 2	0.0 05	0.0 05	0.0 2	0.0 05
qe (cal) (mg/g)	4.20 5	3.7 56	3.6 62	3.8 24	3.6 84	3.7 46	4.2 05	4.2 05	4.2 05	3.6 08	3.7 26	3.6 62	3.8 59	3.7 52	3.9 73	3.8 13	3.7 66	3.8 62	3.9 93	3.6 66	3.7 96
R ²	0.49 6	0.1 11	0.1 97	0.0 09	0.0 02	0.0 08	0.1 78	0.6 81	0.0 3	0.4 44	0.5 4	0.0 01	0.2 11	0.4 9	0.2 74	0.3 11	0.2 85	0.0 61	0.0 28	0.3 42	0.0 3
Pseudo Second order Kinetic Parameters																					
qe (cal) (mg/g)	0.12 5	0.0 44	0.0 49	0.0 45	0.0 43	0.0 48	0.0 45	0.0 42	0.0 44	0.0 41	0.0 43	0.0 41	0.0 46	0.0 43	0.0 5	0.0 44	0.0 48	0.0 47	0.0 5	0.0 42	0.0 45
K ₂ (1/min)	12.0 96	3.6 15	4.9 97	0.4 93	1.6 22	6.5 10	0.9 87	4.5 35	1.0 33	8.9 23	8.1 12	3.5 69	0.1 89	2.7 04	0.3 0.4	24. 61	1.8 7	10 10	1.6 1.6	33 33	2.9 62
R ²	0.96 2	0.9 97	0.9 18	0.9 99	0.9 97	0.9 87	0.9 99	0.9 99	0.9 97	0.9 98	0.9 98	0.9 99	0.9 1	0.9 99	0.9 99	0.9 1	0.9 9	0.9 97	0.9 1	0.9 98	0.9 99
Intra particle diffusion Kinetic Parameters																					
k _{ip} (mg/g/min ^{0.5})	- 0.14 8	- 0.0 11	- 0.0 95	- 0.0 01	- 0.0 01	- 0.0 01	- 0.0 04	- 0.0 21	- 0.0 9	0.0 13	0.0 3	0.0 01	0.0 16	0.0 34	0.0 08	0.0 13	0.0 38	0.0 08	0.0 01	0.0 33	0.0 02
C	13.3 5	22. 84	23. 29	21. 97	23. 3	21. 02	21. 66	22. 49	22. 55	20. 34	21. 82	24. 07	21. 05	21. 75	19. 58	21. 82	23. 16	20. 71	19. 87	22. 26	22. 27
R ²	0.49 8	0.1 11	0.1 74	0.0 1	0.0 02	0.0 01	0.1 79	0.6 81	0.0 3	0.4 44	0.5 4	0.0 01	0.2 1	0.4 87	0.2 75	0.3 11	0.2 6	0.0 6	0.0 28	0.3 45	0.0 3

4.7. Correlation Studies

The adsorption protocol of 45 minutes of contact between adsorbate (metals) and adsorbents (composites) is subjected to correlation studies. The matrix constructed as a result of correlation is shown in Table 4.3a and b.

In case of non-functionalized Fungus biocomposites, it is noted that the correlation between MFPb-MFSiPb, MFPb-MFAICd and MFPb-MFAIPb is found positive and significant at $P > 0.01$ and $P > 0.05$, respectively for the removal of Lead and Cadmium.

On the other hand in functionalized Fungus biocomposites, the significant positive correlation is observed between FMFPb-FMFCd, FMFPb-FMFSiCd, FMFPb-FMFAIPb, FMFCd-FMFSiCd, FMFSiPb-FMFSiCd, FMFSiPb-FMFAIPb, FMFSiCd-FMFAIPb and FMFAIPb-FMFSiAIPb at $P > 0.01$. However, the correlation between FMFPb-FMFSiPb, FMFPb-FMFSiCr, FMFCd-FMFAIPb and FMFSiCr-FMFSiCd are found to be significant at $P > 0.05$ level.

The positive and negative correlation suggests the dependence and non-dependence, respectively of the two components.

It can be seen from the correlation matrix that adsorption of Lead on Macrofungus has a significant positive correlation with composite of Macrofungus with silica (MFSi) and alumina (MFAI). On the other hand, Cadmium uptake is strongly correlated with alumina composite only. The analysis concludes that inorganic moiety of Silica is a good candidate for adsorption of both Pb and Cd, whereas, Alumina for Cd only.

The functionalized Macrofungus composites are generally observed to have more strong associations for the removal of metals in comparison to non-functionalized adsorbents. This is the direct manifestation of functionalization/immobilization of the Macrofungus.

2-tailed significant positive correlations at significant level of 0.01 and 0.05 are observed for a series of composites. It is also interesting to note that a strong paired elemental relationship for Pb-Cd, Pb-Pb, and Cd-Cd (see Table 4.3). This suggests that Pb and Cd adhere competitively the adsorption sites on Macrofungus Functionalized with Ca-alginate, SiO₂ and Al₂O₃. Further, generally less correlation is noted for the removal of Chromium on the composites.

Table 4.3:Correlation Matrix for Removal of Metals at (a) Non-functionalized Composites of Macrofungus (b) Functionalized Composites of Macrofungus

a.

	MFPb	MFCr	MFCd	MFSiPb	MFSiCr	MFSiCd	MFAIPb	MFAICr	MFAICd
MFPb	1								
MFCr	-.038	1							
MFCd	-.160	.445	1						
MFSiPb	.673(**)	.124	-.169	1					
MFSiCr	.093	-.104	-.178	-.180	1				
MFSiCd	.010	-.079	-.062	.215	-.485	1			
MFAIPb	.556(*)	-.102	.014	.002	.108	.024	1		
MFAICr	-.099	-.202	-.239	.171	.000	.241	-.074	1	
MFAICd	.635(**)	-.271	-.376	.252	.065	-.007	.446	.010	1

b.

	FMFPb	FMFCr	FMFCd	FMFSiPb	FMFSiCr	FMFSiCd	FMFAI Pb	FMFAI Cr	FMFAI Cd	FMFSiA IPb	FMFSiA Cr	FMFSiA ICd
FMFPb	1											
FMFCr	.460	1										
FMFCd	.671(**)	.090	1									
FMFSiPb	.721(**)	.303	.474	1								
FMFSiCr	.577(*)	.392	.363	.338	1							
FMFSiCd	.902(**)	.394	.652(**)	.910(**)	.582(*)	1						
FMFAIPb	.835(**)	.263	.569(*)	.925(**)	.363	.925(**)	1					
FMFAICr	.424	.083	.026	.062	.325	.250	.194	1				
FMFAICd	.032	.341	-.360	.153	.154	.045	.123	.115	1			
FMFSiAIPb	.309	.168	-.023	.730(**)	-.138	.472	.623(**)	-.157	.408	1		
FMFSiAICr	.113	.291	-.246	.098	.104	.130	.105	.234	.396	.278	1	
FMFSiAICd	.217	-.077	.420	.450	.213	.454	.338	-.112	-.252	.012	.161	1

Table 4.4: Correlation Matrix of Non-functionalized and Functionalized Biocomposites for Metal Removal (a) Lead (b) Cadmium (c) Chromium

a. Lead

	MF	MFSi	MFAI	FMF	FMFSi	FMFAI	FMFSiAI
MF	1						
MFSi	.673(**)	1					
MFAI	.556(*)	.002	1				
FMF	.556(*)	.588(*)	.209	1			
FMFSi	.925(**)	.703(**)	.596(*)	.721(**)	1		
FMFAI	.754(**)	.635(**)	.549(*)	.835(**)	.925(**)	1	
FMFSiAI	.672(**)	.723(**)	.308	.309	.730(**)	.623(**)	1

b. Cadmium

	MF	MFSi	MFAI	FMF	FMFSi	FMFAI	FMFSiAI
MF	1						
MFSi	-.062	1					
MFAI	-.376	-.007	1				
FMF	-.227	-.004	.723(**)	1			
FMFSi	-.225	.103	.811(**)	.652(**)	1		
FMFAI	.120	.018	-.149	-.360	.045	1	
FMFSiAI	-.192	-.222	.584(*)	.420	.454	-.252	1

c. Chromium

	MF	MFSi	MFAI	FMF	FMFSi	FMFAI	FMFSiAI
MF	1						
MFSi	.673(**)	1					
MFAI	.556(*)	.002	1				
FMF	.556(*)	.588(*)	.209	1			
FMFSi	.925(**)	.703(**)	.596(*)	.721(**)	1		
FMFAI	.754(**)	.635(**)	.549(*)	.835(**)	.925(**)	1	
FMFSiAI	.672(**)	.723(**)	.308	.309	.730(**)	.623(**)	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

- The present study is designed for the development of biocomposite adsorbent from *Pleurotus ostreatus* an edible wild mushroom.
- Calcium alginate and Alumina into the functionalized Macrofungus. Macrofungus and biocomposites were characterized through FTIR, SEM and EDX functional groups –CH, C–O–C (sugar), C=O conjugation and –NH are identified through FTIR. The SEM results confirmed successful functionalization of Macrofungus.
- The fungal fruiting bodies were optimized for metal (Lead, Cadmium and Chromium) removal through batch adsorption. Macrofungus depicts a randomized pattern of adsorption-desorption up to a maximum of 99 %.
- For comparison, prepared biocomposites were also applied for metal removal. Kinetics and isotherms were applied for each metal and each adsorbent. Freundlich and Temkin isotherms fitted best to the adsorption data.
- Further research work can be done on the same organic and inorganic composites for pollutant removal.
- Pb and Cd adhere competitively to the adsorption sites on Macrofungus functionalized with Ca-alginate, SiO₂ and Al₂O₃. Further, generally less correlation is noted for the removal of Chromium on the composites.

FUTURE PERSPECTIVE

The use of microbial biomass has emerged as a new, economical and promising alternative to conventional heavy metal management strategies during the last few decades. Although the mechanism of biosorption is not completely understood but most probably the cell wall components polysaccharides and proteins play a significant role in cation binding.

- In future the underlying mechanism should be explored in depth to enhance the longevity of use. The fungal mycelial mass may also be used for immobilization with calcium alginate or other matrices and explored for its remediation efficiency.
- Further in present research fungal fruiting bodies are used for heavy metal remediation it also suggests the possible use of the fungus for biomonitoring and as bioindicators.
- Removal efficiency of fungus and its composites can be further explored for two metals and three metals (Pb, Cd and Cr) simultaneously.
- Desorption studies of the used adsorbent should be done for the recovery of metals as well as for recycling of the adsorbents.
- In depth study can be designed in future to understand the underlying mechanism of metal removal.
- As the fungus grows on wood not in soil therefore its usage as a bioindicators of atmospheric pollution can further be explored. Its fruiting bodies can also be explored as air pollution remediation technique.

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APPENDICIES

Appendix I - Removal of Metals on Macrofungus as a Function of pH

Lead										
Time (min)	pH⇒Control	2	3	3.5	4	4.5	5	5.5	6	7
Initial	78.664	0.275	35.72	69.13	88.021	85.875	81.488	46.515	31.527	50.529
3	78.907	16.501	40.49	99.61	91.530	85.076	87.114	45.912	41.168	51.743
6	72.492	17.290	36.55	94.42	99.344	84.507	88.384	51.698	38.420	46.918
9	83.886	15.389	40.60	99.29	56.824	85.452	49.199	51.544	40.996	52.367
12	82.797	22.175	43.52	97.88	47.818	63.845	53.020	51.120	27.974	52.341
15	81.086	23.372	38.03	93.32	53.477	88.245	52.353	68.298	39.744	48.930
18	78.599	26.710	31.75	98.36	58.589	48.860	45.867	59.500	41.699	52.930
21	82.088	25.386	41.51	92.25	55.193	56.088	43.630	83.327	44.515	45.013
24	90.896	21.389	38.13	98.63	63.398	49.638	47.270	88.394	44.183	52.162
27	84.228	39.885	19.11	95.06	42.287	61.202	54.866	89.538	43.788	53.574
30	84.681	27.748	43.21	96.60	41.052	58.360	54.380	61.756	40.077	48.730
33	87.703	26.915	31.43	96.35	54.973	62.402	55.139	60.855	45.770	71.968
36	81.407	26.888	29.84	97.11	46.620	64.608	46.046	66.023	45.291	54.478
39	72.087	25.562	41.48	98.67	53.965	64.799	61.049	64.726	54.647	52.688
42	72.076	91.865	42.42	98.44	47.548	64.273	57.497	67.387	69.291	52.645
45	71.930	25.096	39.36	96.11	49.668	55.478	60.408	71.065	69.296	37.208
Cadmium										
	Control	2	3	3.5	4	4.5	5	6	7	
Initial	53.902	90.954	95.277	94.633	94.378	77.282	50.300	52.280	95.346	
3	47.743	91.051	91.471	94.662	94.186	75.630	43.659	71.320	95.926	
6	56.843	90.428	91.552	94.587	94.979	88.517	53.470	65.600	95.294	
9	53.902	91.255	91.748	94.670	94.936	87.856	50.300	63.160	99.208	
12	33.755	91.007	91.735	93.339	94.693	78.686	28.578	61.720	96.467	
15	63.241	91.373	99.864	93.895	93.389	87.113	60.368	64.520	96.042	
18	58.870	91.500	90.516	94.479	93.700	86.039	55.656	72.400	96.166	
21	73.613	91.072	91.935	94.347	94.020	85.708	71.551	73.080	96.299	
24	59.943	90.827	91.449	94.355	94.621	76.621	56.812	67.680	96.851	
27	45.239	91.100	91.829	94.384	95.665	85.048	40.960	71.840	95.738	
30	28.112	99.804	92.387	88.598	95.107	85.213	22.494	30.400	99.144	
33	55.293	92.070	91.513	96.549	95.124	87.278	51.799	60.440	93.293	
36	34.470	98.301	93.393	95.185	94.241	79.760	29.349	54.000	96.066	
39	12.017	91.748	92.600	90.336	96.070	87.691	5.141	65.640	95.946	
42	30.178	90.689	91.287	91.738	95.904	88.104	24.722	73.120	96.126	

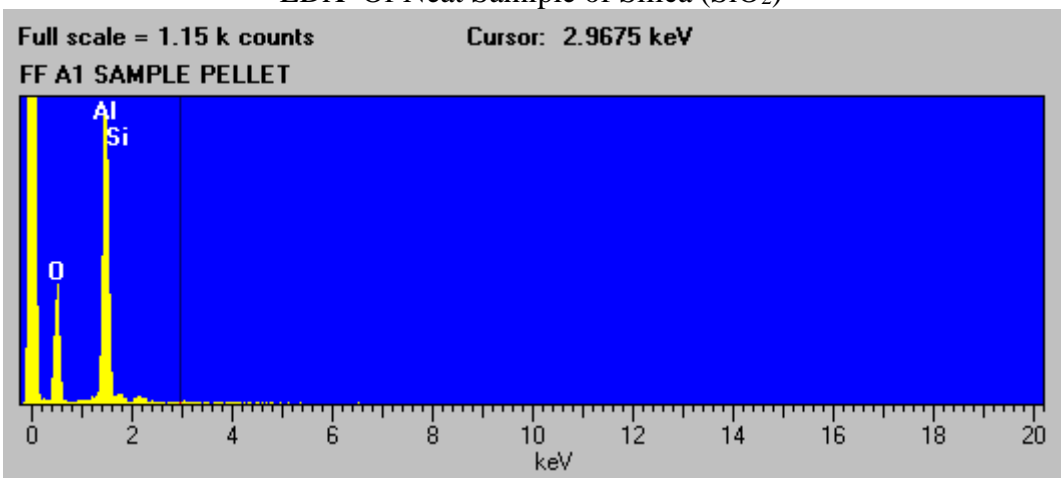
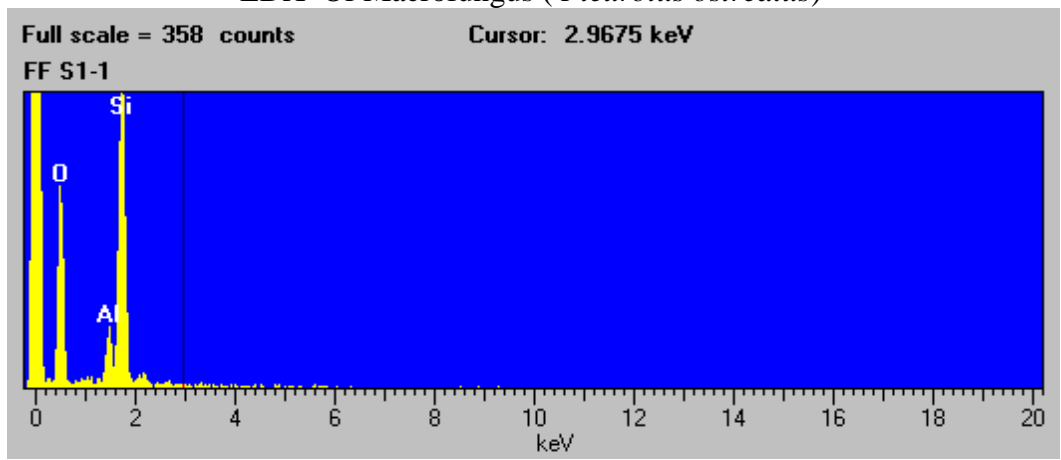
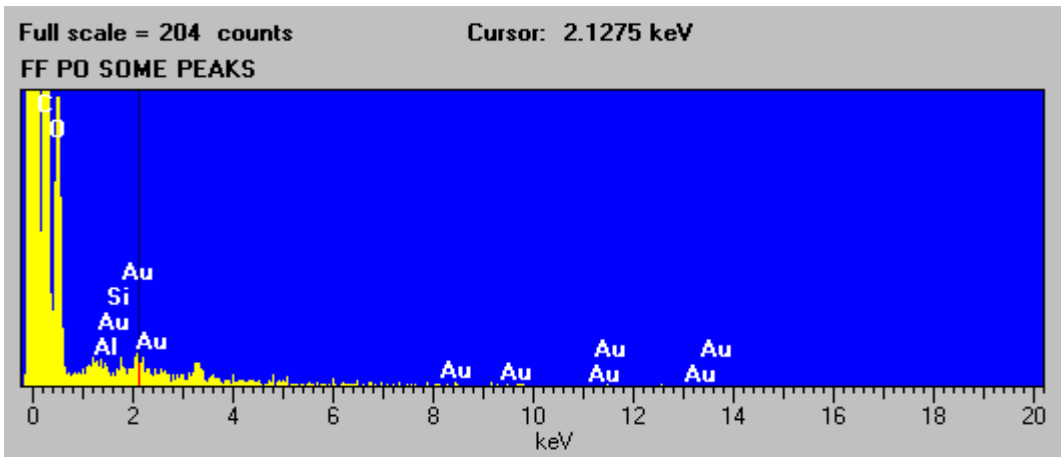
Chromium									
	2	3	3.5	4	4.5	5	5.5	6	7
Initial	96.97596	31.644	63.156	18.154	18.154	79.310	96.929	53.348	7.701
3	99.47944	74.142	59.441	26.576	26.576	79.835	99.475	4.975	12.203
6	98.04888	77.015	65.607	42.692	42.692	54.942	98.020	5.862	16.644
9	96.37989	82.921	63.424	56.195	56.195	55.023	96.323	10.100	17.190
12	93.24061	81.125	62.735	18.134	18.134	56.114	93.130	4.538	24.003
15	89.18736	79.050	66.028	16.156	16.156	41.122	89.008	36.600	29.699
18	44.28373	84.517	61.049	25.020	25.020	71.187	43.344	10.100	33.350
21	88.5913	95.451	57.717	30.230	30.230	78.987	88.402	14.515	38.936
24	86.56467	78.691	59.173	41.100	41.100	84.563	86.341	19.499	36.218
27	86.00834	85.874	42.627	38.656	38.656	91.837	85.775	13.239	41.906
30	85.53149	83.160	22.903	54.744	54.744	88.766	85.291	19.410	44.351
33	80.72323	75.898	63.960	50.330	50.330	91.271	80.401	22.377	48.442
36	84.45857	78.851	58.445	53.268	53.268	94.787	84.199	22.447	52.777
39	83.90225	75.060	66.909	53.240	53.240	93.656	83.634	25.507	56.668
42	77.34552	86.872	71.582	58.494	58.494	93.494	76.966	31.029	49.619
45	81.20008	91.141	70.969	61.067	61.067	95.838	80.886	32.530	56.590

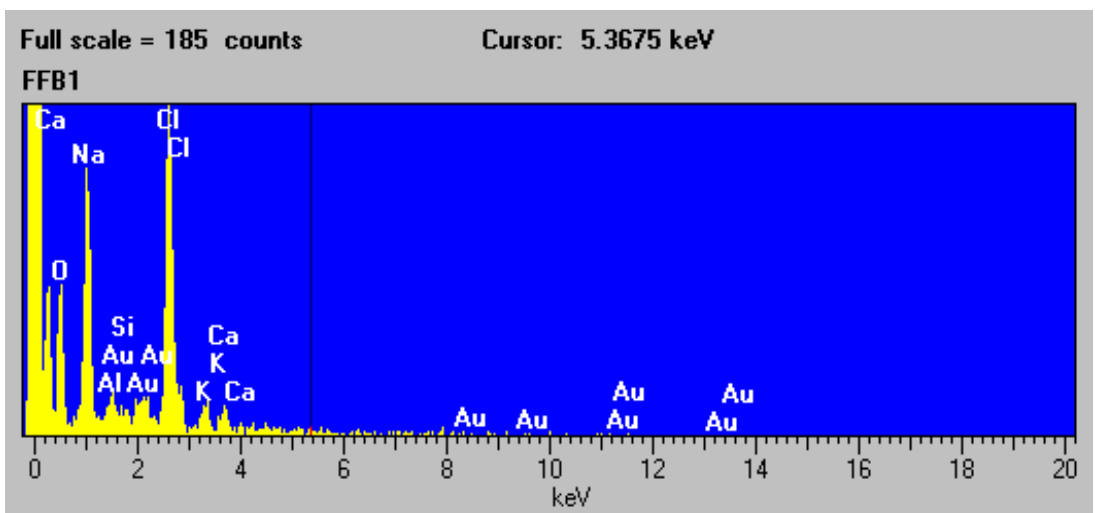
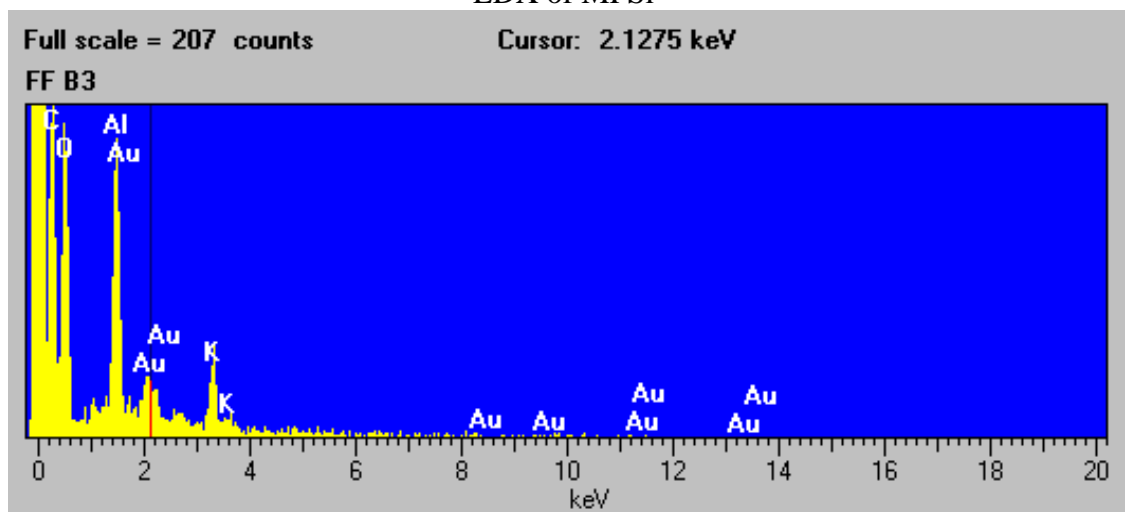
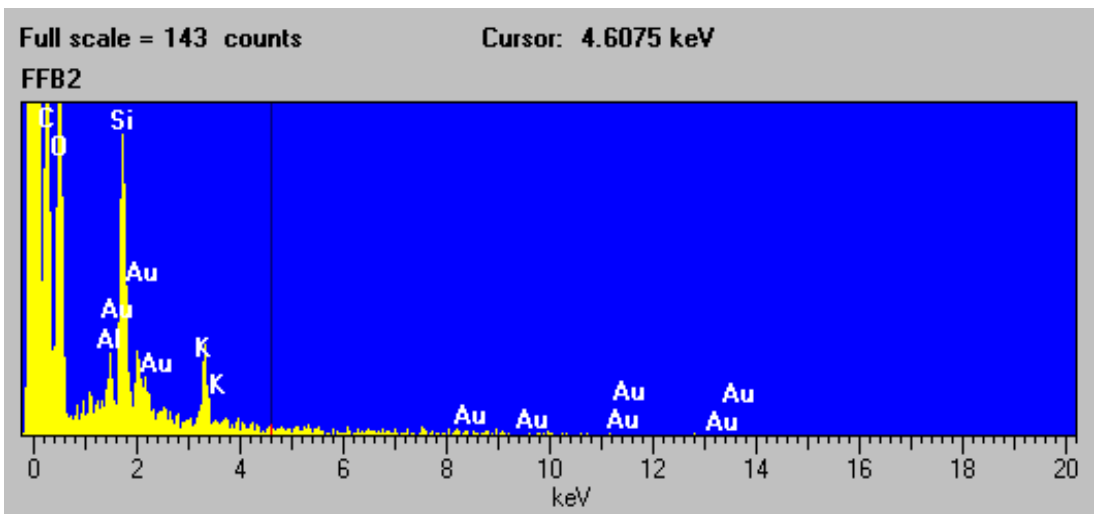
Appendix II - Removal of Metals on Biocomposites at optimum pH

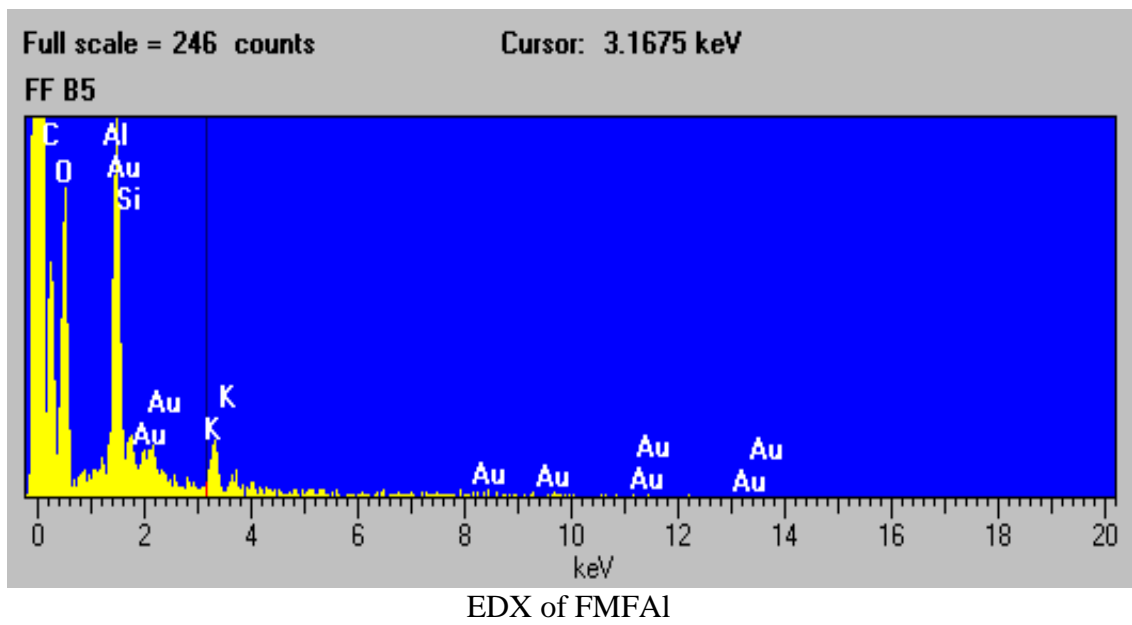
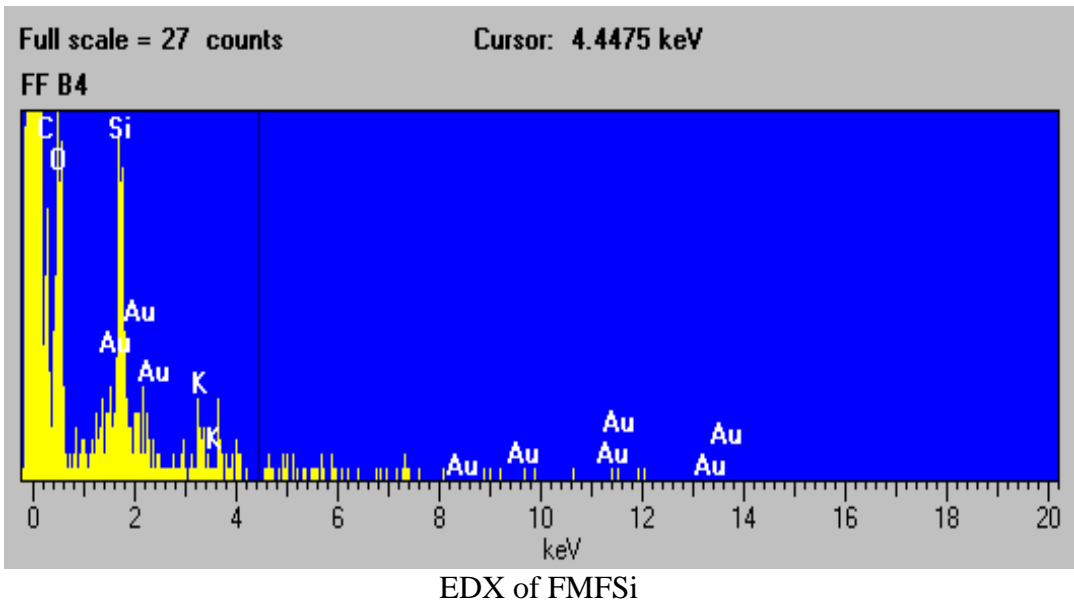
Time (min)	FMF	MFSi	MFAI	FMFSi	FMFAI	FMFSiAI
	Lead					
Initial	67.88	90.31	89.44	82.51	85.61	86.593
3	69.62	91.47	91.62	90.48	88.60	88.450
6	82.25	92.16	91.41	90.89	90.79	89.085
9	89.70	92.68	90.01	90.59	89.41	88.272
12	97.22	92.95	90.00	91.30	90.58	88.641
15	97.50	92.23	90.36	91.42	91.10	88.583
18	97.66	92.20	90.94	91.16	90.04	88.450
21	98.69	92.20	91.34	91.37	91.03	88.312
24	96.10	91.98	91.09	91.45	90.57	88.481
27	94.82	91.54	90.66	91.09	90.52	88.192
30	95.43	91.93	91.09	91.32	90.86	88.276
33	94.66	91.80	91.37	91.35	90.86	87.606
36	97.39	91.28	91.80	91.39	90.90	87.761
39	96.20	92.28	91.07	90.93	90.68	87.912
42	95.21	91.66	91.05	90.83	90.56	87.859
45	95.40	92.27	90.90	91.22	90.26	88.001

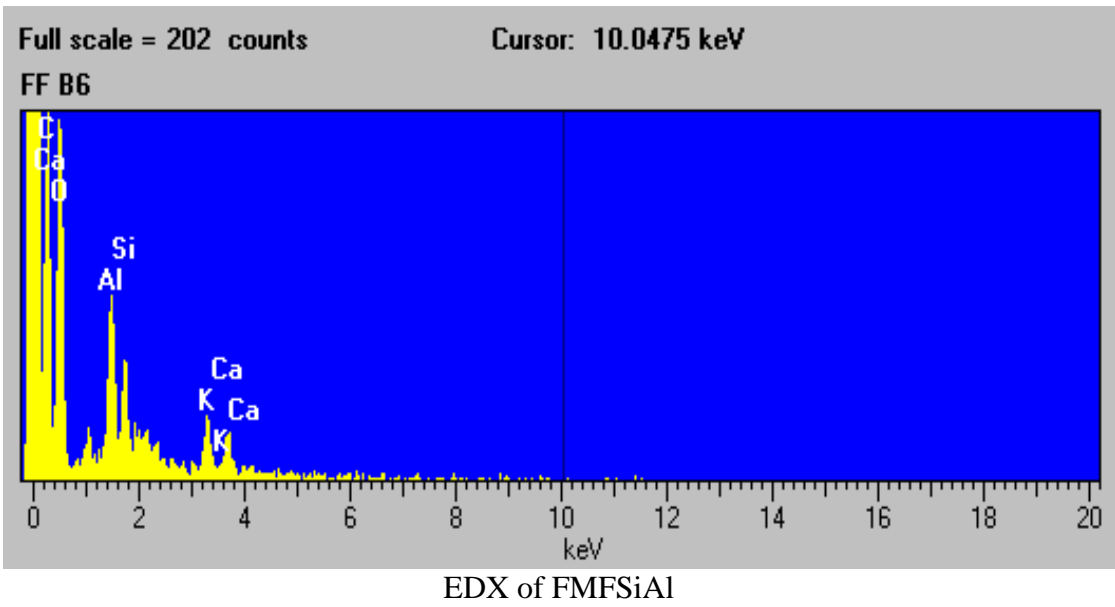
Cadmium						
Initial	85.952	94.528	92.211	84.574	91.72	87.74
3	87.048	94.572	94.464	90.862	95.25	98.59
6	88.032	94.988	94.139	92.308	94.90	88.71
9	88.632	95.077	94.685	93.880	95.81	89.35
12	89.760	94.230	95.069	93.813	96.97	88.55
15	90.320	94.890	95.699	95.360	96.98	92.58
18	91.584	95.309	97.468	95.201	96.88	93.08
21	90.344	95.346	94.514	95.502	97.13	95.00
24	86.472	94.092	95.891	95.686	97.07	93.54
27	92.184	95.167	97.038	95.619	97.88	93.87
30	89.328	94.625	97.818	95.443	97.45	95.96
33	90.248	95.183	97.000	95.619	97.88	93.87
36	93.416	94.377	96.938	95.435	87.10	96.81
39	92.760	98.860	97.025	96.263	88.51	93.31
42	93.528	90.375	97.242	95.268	90.12	97.24
45	90.768	94.691	97.076	95.343	83.52	95.87
Chromium						
Initial	94.50	94.28	86.30	85.09	82.940	88.798
3	95.38	94.33	85.82	84.58	79.210	89.207
6	93.10	94.76	86.12	84.73	80.917	88.678
9	97.96	94.85	85.34	87.41	80.474	88.117
12	98.06	93.97	89.50	84.90	83.208	87.764
15	99.03	94.66	86.96	86.05	82.363	90.068
18	96.58	95.10	87.07	86.51	84.449	89.615
21	97.87	95.13	92.27	86.70	82.552	90.769
24	99.19	95.13	78.86	86.56	84.820	90.609
27	97.33	95.13	88.96	87.57	82.315	89.631
30	93.13	94.95	88.23	86.63	84.473	89.187
33	97.38	94.38	86.42	87.84	84.970	87.925
36	94.78	94.96	85.54	86.04	84.101	88.349
39	96.62	94.12	88.37	86.03	83.627	88.650
42	96.59	98.81	86.44	86.23	78.412	87.776
45	95.58	89.94	85.97	86.72	82.821	88.562

Appendix II EDX OF Pristine Samples and Developed Biocomposites

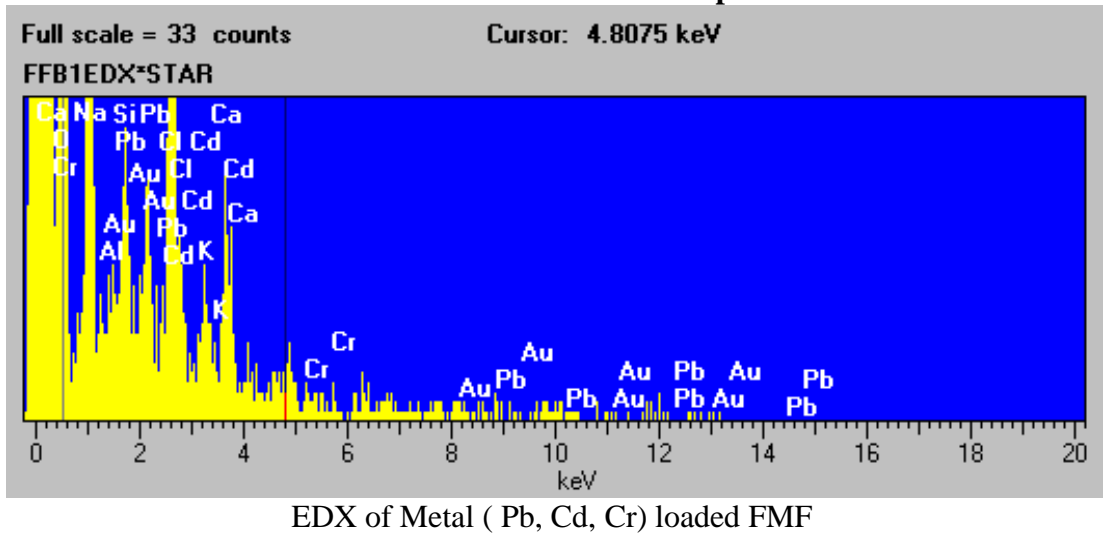


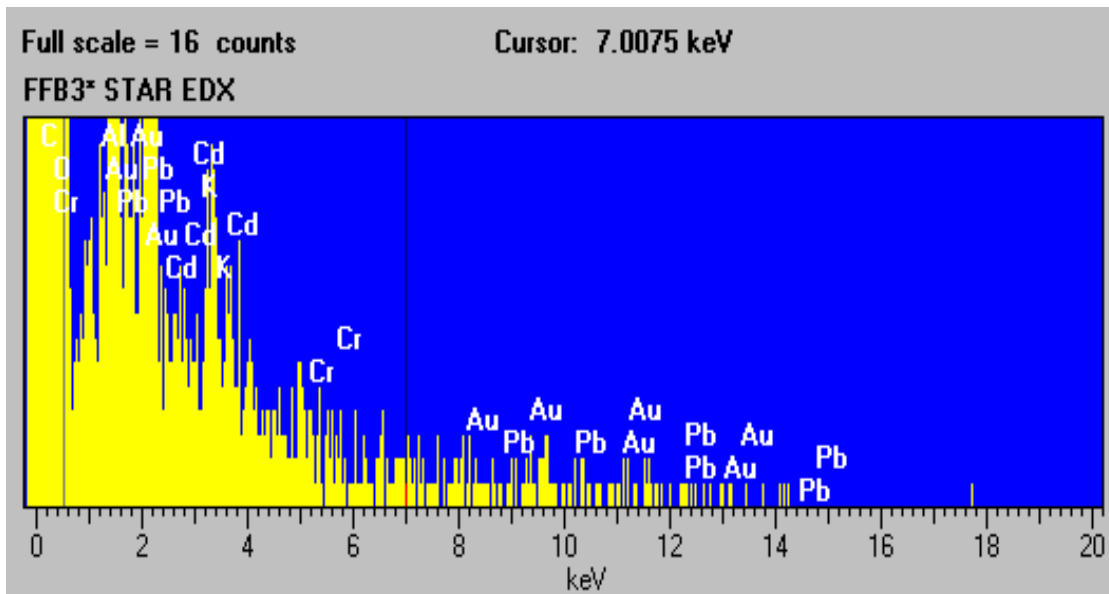
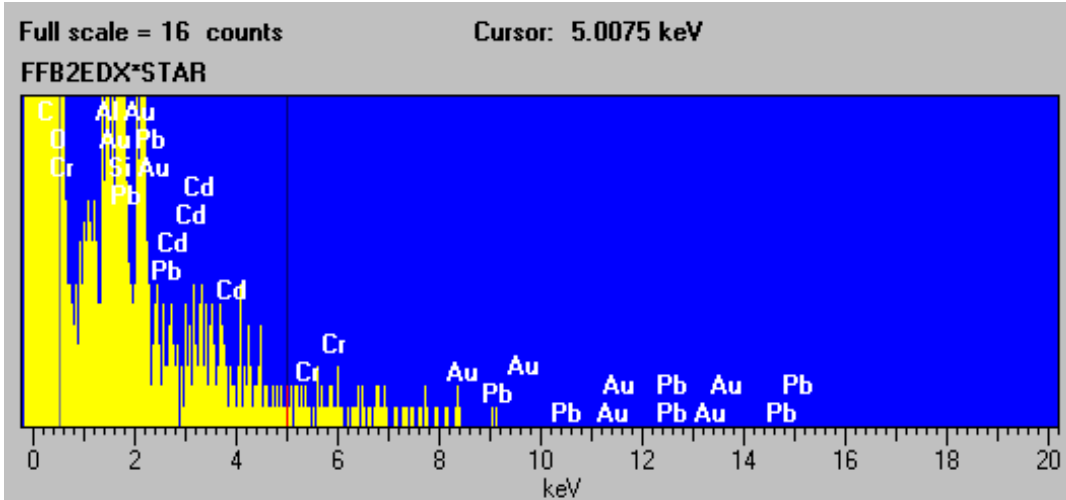






EDX of Metal Loaded Biocomposites

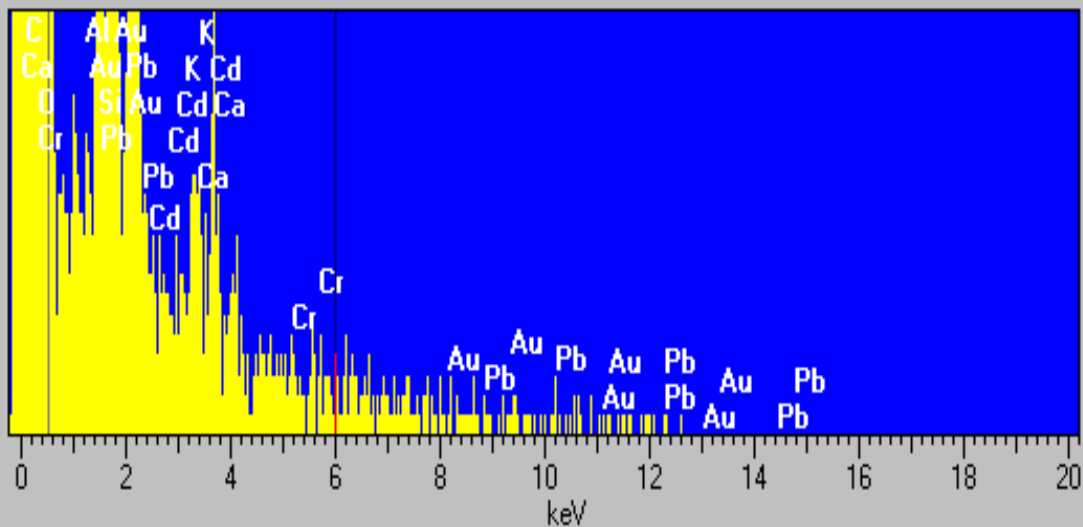




Full scale = 21 counts

Cursor: 6.0075 keV

FFB4*EDX STAR

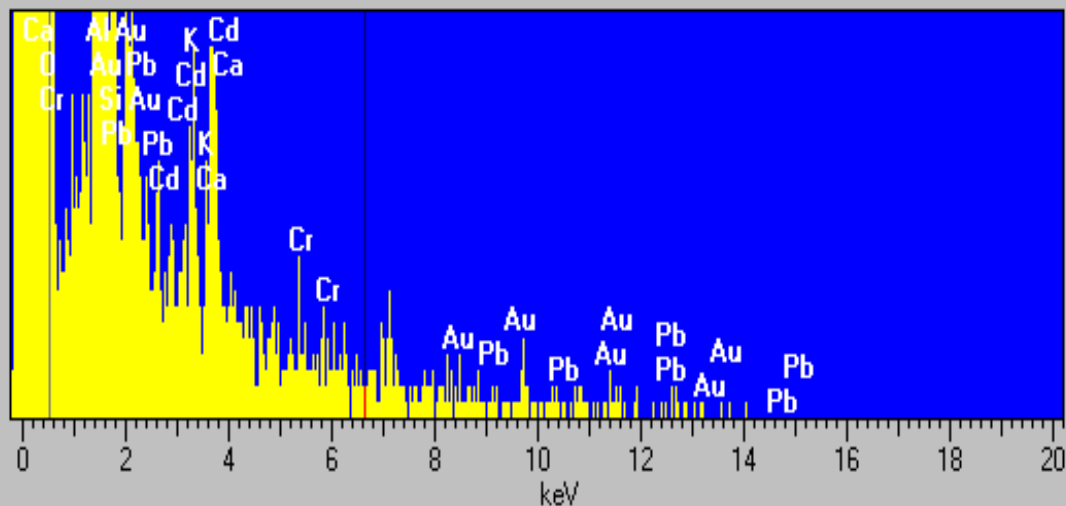


EDX of Metal (Pb, Cd, Cr) loaded FMFSi

Full scale = 25 counts

Cursor: 6.6475 keV

FFB5*STAR EDX

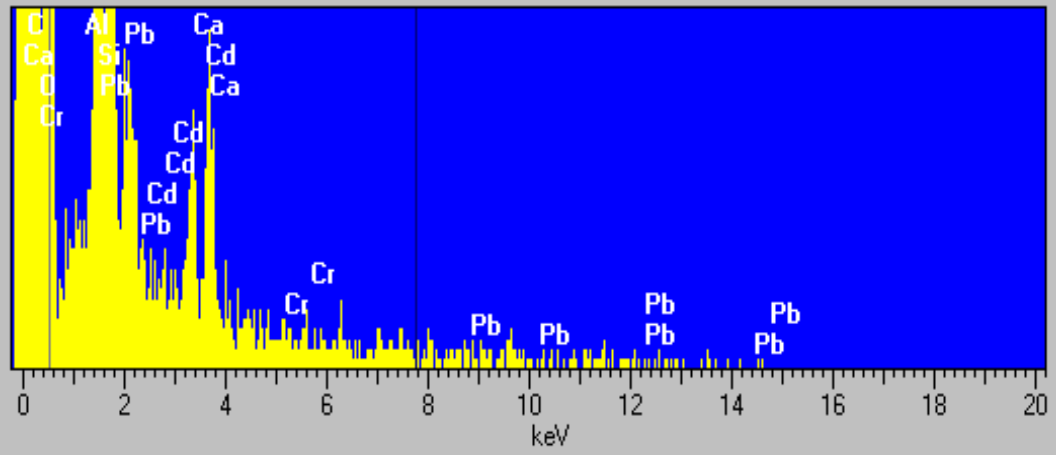


EDX of Metal (Pb, Cd, Cr) loaded FMFAI

Full scale = 36 counts

Cursor: 7.7675 keV

FFB6*STAR EDX



EDX of Metal (Pb, Cd, Cr) loaded FMFSiAl