A REPORT ON BIOREMEDIATION OF CD (II) POLLUTION USING *Lathyrus sativus* HUSK

(A REFERENCE BOOK)

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Introduction:

Environmental pollution is becoming of greater and greater concern with the increase in human population along with the rise in the production of industrial and domestic wastes. Unlimited exploitation of nature by man disturbs the delicate ecological balance of the biosphere. By definition, pollution is an undesirable change in the physical, chemical or biological characteristics of air, water and soil that may affect the life or create a potential health hazard of any living organisms. Pollution in its everyday manifestations, however, cannot be completely eliminated [1]. But it can be controlled by judicious treatment procedures. The problem rapidly escalates in severity when the rate of pollution emission exceeds the capacity of the environment to assimilate them.

A pollution may thus include and solid, liquid or gaseous substance, released on such concentration as may be or tend to be injurious to the environment. Within the array of pollution, chemical pollution occupies an important position. The chemical pollutants are widely classified into organic and inorganic pollutants. The first group includes polychlorinated biphenyls (PCBs), oils and many pesticides whereas the second group includes salts, nitrates and metals. Metals, which are a major subcategory of inorganic pollutants, may also exist as organ metallic pollutants [2]. Environmental contamination by toxic metals is a serious problem due to their incremental accumulation in the food chain and continued persistence in the ecosystem [3, 4]. Unlike organic chemicals, metals are elemental and do not break down. Among the metals, ‘heavy metals’ pose special environmental concern and deserves intensive attention.

‘Heavy metals’ is a general collective term applying to the group of metals and metalloids with an atomic density greater than 6g/cc [2]. It is usually applied to the elements such as Cd, Cr, Cu, Hg, Ni, Pb and Zn, which are commonly associated with pollution and toxicity problems. Unlike most organic pollutants, heavy metals occur naturally in rock forming and are minerals and so there is a range of normal background concentrations of these elements in soils, sediments, waters and living organisms. Pollution occurs when the concentration of these metals highly exceeds the normal background level. Therefore, presence of the metal is insufficient evidence of pollution: the relative concentration is all important [1, 2].

Apart from aerosols in the atmosphere and direct effluent discharges into waters, the concentrations of the heavy metals available to terrestrial, aquatic and marine organisms (i.e. their bioavailability) is determined by the solubilization and release of metals from rock forming minerals and the adsorption and precipitation reactions which occur in soils and sediments [2]. Although heavy metals differ largely in their chemical properties they are used widely in electronics, machines and other requirements of everyday life as well as ‘high – tech’ applications. Consequently they tend to reach the environment from various anthropogenic sources as well as natural geochemical process. Some of the elements (Cu, Zn, Fe, Mn, etc) in the ‘heavy metals’ groups are required by most living organisms in small but critical concentration for normal healthy growth and known as micronutrients. But some other elements (As, Cd, Pb, Hg, etc). With no known essential biochemical function cause toxicity at concentration which exceed the tolerance of the organisms but do not cause deficiency disorder at low concentrations like the micronutrients.

Some concerns associated with metal pollution are as follows:

- Pollution in fresh waters is a concern because natural metal levels in these water bodies are often low and many lakes have small volumes or few outlets to displace polluting metals.
- Pollution of coastal waters is a major concern as these are increasingly contaminated by human activities.
- Older cities, which traditionally have more metal processing facilities than rural environments, are often highly polluted with metals.
- Metal contamination of agriculture soils presents serious problems. Above the loading rate, the soil cannot safely be used to grow crops. In Japan, nearly 10% of the paddy land can no longer be used because of cadmium and lead contamination [1, 2].
- Many metals (e.g. aluminum) are more soluble in acidified soils and being mobilized by acid can run off into nearby water bodies and can adversely affect aquatic life [1].
- One concern centering around these metals is that living organisms concentrate them to levels much higher than found in the environment. People with poor nutrition or those eating large amounts of a contaminated food are most vulnerable to the adverse effects of metal exposure. Children, even those who are well nourished, are move vulnerable than adults because they absorb most of the metals that they ingest across the intestine [2].

- The heavy metals lead, cadmium and mercury are 3 of the 10 most common pollutants found in the hazardous waste sites. Metal contaminated soil can be remediated, but at heavy cost, clean up of large areas may be impossible [1].

Presence of those toxic heavy metal ions in surface water continues to be one of the most pervasive environmental issues of the present items. Among the mental ions polluting surface water to a larger extent after being released as industrial effluent. Cd ions occupy an important position. Increasing attention is therefore being paid on the development of know how for removal of Cd ions from metal bearing effluents before their discharge into water bodies and natural streams. Methods to detect the cadmium contamination in waste waters and their impact on environment are improving day by day.

Cadmium as a pollutant:

Cadmium is one of the non essential toxic metals that cause toxicity at concentration. Which exceeds the tolerable limits of the living organisms [1, 2, 3]. Cadmium was not discovered until 1817 and has been heavily mined only since the end of Second World War [1]. It was discovered by Stromeyer (1817) from an impurity in Zinc carbonate. This transitional metal has much similarity with zinc. Cadmium infiltrates into the environment form both natural and anthropogenic sources.

Source of cadmium:

Although heavy metals like cadmium is ubiquitous in most natural materials. Various anthropogenic activities are the most significant courses of cadmium to the environment.

Natural sources:

Geochemically cadmium occurs as ‘impurity’ isomorphously substituted for various microelement constituents of the crystal lattice of many primary minerals [2]. The natural enrichment of Cd^{2+} ions in the soil gives rise to harmful effects in living organisms. There is also normal background of cadmium in atmosphere and hydrosphere. The typical concentration (in ug/1) of cadmium in the earth’s cruse and major rock types (from alloway, 1990) as well as in hydrosphere (from Bowen, 1979) are as follow:

Natural sources	**Concentrations (ug/1)**
Earth’s cruse	0.1
Igneous rocks	
Ultramafic	0.12
Mafic	0.13
Granite	0.09
Sedimentary rocks	
Limestone	0.28
Sandstone	0.05
Shales / Clay	0.22
Hydrosphere	

Fresh water	0.01-3
Sea water	<0.01-4

Anthropogenic sources:

Among the man-made sources, metalliferous mining is one of the prominent sources of cadmium pollution. The common are mineral of cadmium is ZnS (Peters, 1978). Zinc ores often contains relatively high concentrations of cadmium (<5%) and so Zinc production can give rise to significant environmental pollution with Cd. Cadmium also occurs as associated metals of the minerals of many other metals like Cu, Pb and Zn. Pacyna (1987) estimated that primary non-ferrous metal production gave rise to atmosphere emission of 1630t Cd/yr [2]. As with lead and a number of other metals, Cd is released into the air by mining and smelring operations and contaminates the environment in the vicinity of the mines and smelters. Anomalously high cadmium concentrations were found in soils, surface water and vegetations up to 40km downwind from historic smelting activities in the lower Swansea Valley in South Wales and 15km from the Avon mouth Pb-Zn smelter near Bristol, in U.K. [5].

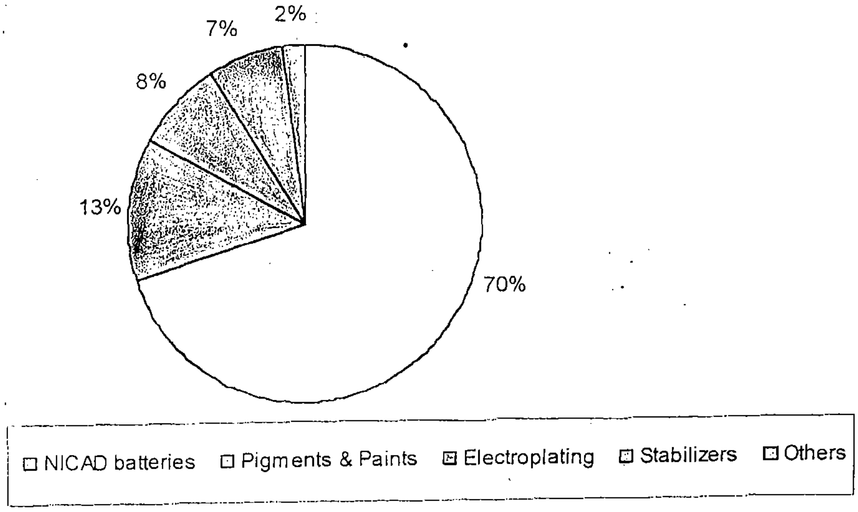
Agriculture constitutes one of the very important non point sources of metal pollutants like cadmium. It may exist as:

- Impurities in fertilizers particularly in phosphatic fertilizers.
- Composts and manures
- Sewage sludge.
- Corrosion of metal objects e.g. galvanized metal roofs and wire fences.

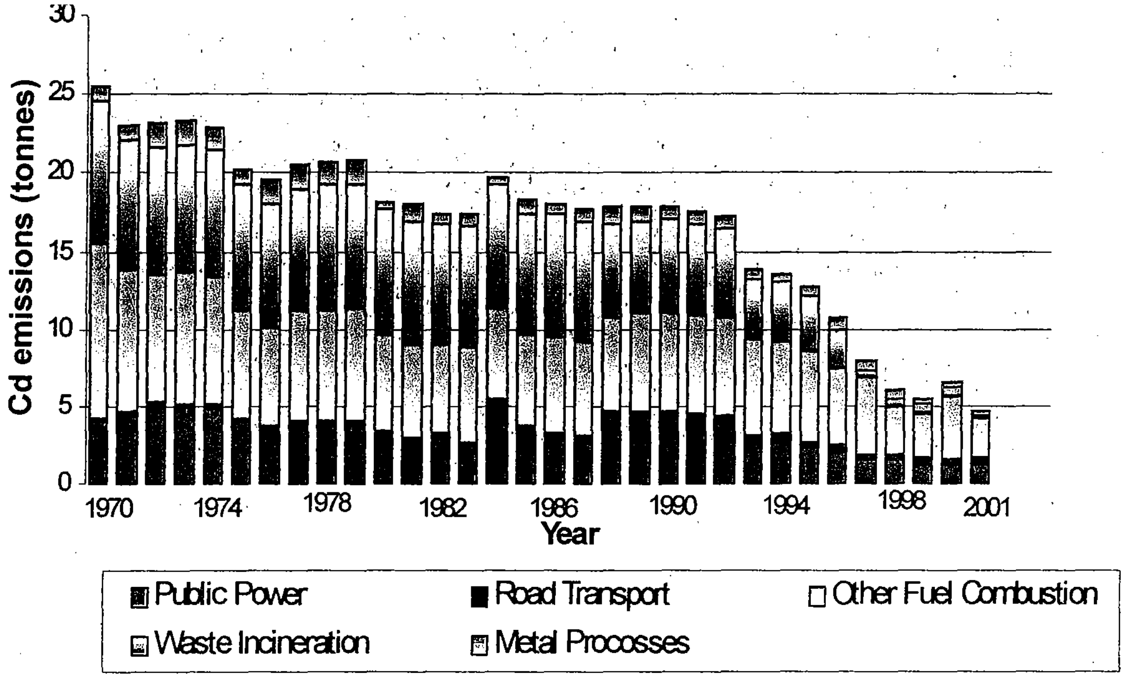
Cadmium is also found in fossil fuels, which are either emitted into the environment as particles during combustion or accumulates in ash which may itself be transported and contaminate soils or waters, or may be leached in situ. Cd is also dispersed into the environment in leachates from landfills, which pollute solids, ground and surface waters, and in fumes from incinerators. Although present in many sludges in quite low concentrations (<10ug/g), cadmium is relatively easily taken up by food crops, especially leafy vegetables, and enters the human diet [2]. The other significant anthropogenic source of cadmium includes:

1. Nickel Cadmium rechargeable batteries (Nicad batteries).
2. Pigments and paints
3. Polymer stabilizers
4. Printing and graphics
5. Electroplating

The above mentioned industries rank among the principle sources of cadmium in the municipal solid wastes. But the highest emission occurs from the batteries, followed by pigments and paints, electroplating, stabilizers and others. Contribution of these industries in cadmium emission (in %) is shown in the following chart:



The emission of cadmium in MSW from household batteries was 930 tons in 1986, which has been increased to 2035 tons in 2000 [1]. But, in the case of plastics, however, the emission of cadmium has been reduced from 502 tons in 1986 to 380 tons in 2000 (Hill, 2000). Although cadmium is no longer added to disposable batteries, it is used in Nicad rechargeable batteries [1, 5]. As with disposal batteries, the final fate of rechargeable batteries is to be discarded. Thus, Nicad batteries continue to be the greatest source of cadmium in the solid waste stream, and, as the above data mentioned, the amount continues to increase. However, Cd levels in the environment reached a peak in the 1960s. Since then, the levels have been constantly decreasing due to improved technology for the production, use and disposal of Cd (as shown in Fig. 2):



Guideline values for cadmium:

Legislation and guidelines to control the emission of heavy metals like Cd are becoming more than comprehensive day by day. Earlier estimates of anthropogenic cadmium emission vs. natural cadmium emission had indicated approximately 8000 to 10, 000 metric tons / year for anthropogenic emission compared to 800 to 1000 metric tons / year for natural cadmium emission (WHO, 1992). The guidelines value for cadmium in drinking water introduced by WHO is 3ug/1 and that introduced by EPA and EC Directive is 5ug.1. Under the European community directive 86/278, the maximum permissible concentration of Cd in sludged soil is 3ug/g. The food and Drug Administration (FDA) limits Cd food colors to 15 ppm. The standard limit for Cd emission in air has been recommended to be 3mg/Nm^3 by environmental pollution control (air impurities) regulations, 2000. The occupational safety and health administration (OSHA) limits cadmium emission in workplace air to 100ug/m^3 as Cd fumes and 200ug/m^3 as Cd dust. According to the stack gas emission standards, (Environmental quality regulations, 1978) the standard limit for Cd is 0.015 g/Nm^3. Ministry of environment and forest (New Delhi, 25th September, 2000) have introduced the municipal solid wastes (management and handling) rules, 2000. According to this rule the concentration of Cd not to be exceeded in order to ensure safe application of compost is 5mg/kg dry basis. In case of disposal to treated leachates also, the rule limits the concentration of Cd in inland surface water to 2.0mg/1 and in pubic sewers to 1.0mg/1.

Route of exposure and toxic effects of cadmium:

More than 90% of the average non smoker's exposure to cadmium is though food [1]. She 11 fish concentrates cadmium ad consumption of scallops and oysters can be a major exposure source for people who ear them. Fish concentrates cadmium to a lesser extent and are a lower source of exposure. Liver and kidney concentrate cadmium and contribute to the exposure of those who consume these organs [1, 2, and 3]. Instantaneous poisoning effect occurs when some sour food articles such as lemonade, etc. are taken from a utensil made of cadmium.

Cd becomes very volatile above 400oC and hence is likely to be dispersed as an aerosol when mixtures of metals containing Cd are heated or case [2]. High concentrations of inhaled Cd aerosols can cause emphysema and related acute lung conditions.

High cadmium levels in soil and surface water present special concerns because plants take up Cd more readily than other metals. Cd has a higher transfer coefficient (1-10) which is a reflection of its relatively poor sorption in soil [2]. Because tobacco plants concentrate cadmium, smoking a pack of cigarettes a day can double a person's exposure [1]. Worse, an adult absorbs more than 90% of the cadmium inhaled though smoking as compared to only 5% of the cadmium ingested in food. The greatest route of exposure for workers with occupational exposure is inhalation.

Phytotxicity:

Cd is one of the most toxic metals for both higher plants and several microorganisms. In leaves the normal range of cadmium concentration is 0.1ug/g to 2.4ug/g. Toxicity arises when the concentration rises up to 5ug/g to 30ug/g [2]. The toxicity also depends on soil factors, such as pH, the plant genotype, the condition under which it is growing and also toleration of individual plant species. The biochemical processes in plants, which are affected by Cd toxicity are as follows:

1. Changes in the permeability of cell membranes
2. Bonding to sulphydryl groups
3. Inhibition of enzymes
4. Photosynthesis
5. Transpiration
6. Respiration
7. Chlorosis

Mammalian toxicity:

Cd toxicity is affected by considerable variation in toleration of individuals and, in the case if diets, in the composition of the diets. Chemical form of the element will also affect its toxicity. Investigations conducted around the world show that, for the general population, the average daily Cd intake from all sources is very low and at the lower end of the total range of 10-25 ug/day. The tolerable daily cadmium intake established by WHO is 60-70 ug/day for adults. Acute lethal dose of Cd injected in to the peritoneum of the mammals has been estimated to be 1.3mg/kg of the body weight (Bowen, 1979). The metal was injected to the peritoneum to avoid absorption through the digestive tract. If added in human diet the toxic dose of cadmium has been found to be 3-330 mg/day (Bowen, 1979). The cancer slope factor for cadmium is 6.1mg/kg body weight / day (Hill, 1999).

Homeostatic mechanisms in animals frequently involve special proteins, metallothioneins, which binds with the metals like cadmium and render them relatively inactive [2]. Cadmium bio accumulates in the kidney and the amount of Cd stores in this organ increases with age. In some individuals, if concentrates to levels not much lower than those known to damage the kidney [1]. For those with high occupational exposure to cadmium, kidney damage has been the most prevalent chronic effect [2].

Because Cd affect calcium metabolism, bone degradation has also been observed. Cadmium can also accumulate to high levels in the liver. The symptoms are nausea, vomiting, loose stool and weakness. The symptoms will appears within 10 minutes and subside generally within 24 hours. High concentration of inhaled cadmium aerosols can cause emphysema and related acute lung conditions. In rodents, cadmium shows numerous toxic effects including birth defects, and is a carcinogen [2].

Example of cadmium poisoning:

A serious case of poisoning occurred in Jinstu Valley in the Toyama prefecture in Japan, where Pb-Zn mining had caused widespread Cd and Zn contamination of the alluvial soils, most of which were used for paddy rice production [1, 2, 5]. The farmers mainly live on rice grown in the contaminated paddies and also relied on the metal polluted rivers for their drinking water. After the second worlds war, it was found that more than 200 elderly woman having several children, had developed kidney damage and skeletal deformities. It was called 'itai'itai' disease.

The Cd toxicity was exacerbated by the deficiency of calcium, vitamin D, protein diet and birth of several children. It was estimated that the people in the valley has a cadmium intake of around 600ug Cd/day, which is around ten times greater than the maximum tolerable intake of 60-70 ug/day [2]. A survey of paddy soils in the whole of Japan revealed that 9.5% of the area was significantly contaminated with Cd, with a further 3.2% of upland rice growing soils and 7.5% of orchard soils. The source of Cd in these soils is probably phosphatic fertilizers and industrial / mining pollution.

Conventional methods of cadmium removal:

Discharge of Cd^{2+} ions to the aquatic environment has been a major cause of concern and the treatment of these wastes has consequently attracted considerable attention. Where the metal is substantially in solution there are various traditional techniques for separation or concentration of the metal so that a high quality treated effluent may be obtained. The common conventional treatments include ion exchanges, lime precipitation, electrolysis, reverse osmosis and many such other techniques.

Ion exchange is a chemical treatment process used to remove dissolved ionic species from contaminated aqueous streams. Treatment for both anionic and avionic contaminants can be effected by ion exchange processes. Ion exchanges are insoluble high molecular weight polyelectrolytes that have fixed ionic groups attached to a solid matrix [5]. Both natural and synthetic ion exchangers are available. However, due to their greater stability, higher exchange capacity and greater homogeneity of their exchange properties, synthetic ion exchange materials are predominantly used today. Synthetic ion exchange are generally polymeric materials (resins) that have been chemically treated to render them insoluble, and to exhibit ion exchange capacity polymers of styrene and divinylbenzene (DVB) are the most common synthetic ion exchange materials.

Molecular filtration is divided into two categories. Ultration (UF) and reverse osmosis (RO) processes [5]. RO membranes operate with molecular cutoffs that are much smaller than UF membranes (100-200 Da compared with 2-1000 mDa), therefore the RO membrane is capable of retaining more inorganic solutes. RO membranes operate with Tran’s membrane pressure of up to 500 psi. Often when reverse osmosis is used, upstream UF is provided as a pretreatment for RO. The main operational problems associated with membrane process are chemical and biological fouling of the membrane, and particularly with RO. Membrane deterioration. The RO process has been profoundly used on effluents form electroplating in the electronic industry.

The most appropriate method of treatment depends on the concentration of Cd^{2+} ions solution. pH other associated constituents and the desired effluent standard [2,5]. The technique most commonly employed in treating metal processing wastes is precipitation using pH adjustment. Removal of Cd^{2+} ions by chemical precipitation with lime or caustic soda is one of the common conventional treatments. But in this process the recovery of the metla or water is nto possible [2]. Moreover, chemical addition can be expensive, often requires pH correction, and may produce large quantities of sludge with a disposal problem. For these reasons chemical enhancement of primary sedimentation is rarely practiced at plants treating domestic wastes. Nevertheless for many toxic industrial waste waters this is an attractive treatment option since it enables industry to avoid secondary biological treatment and enables the wastes to be discharged to a sewer, estuary or the sea.

Electrolysis is another most commonly used technique to remove dissolved metallic ions (like Cd^{2+}) from wastewater. When an inorganic salt is dissolved in a water solution it ionizes to produce positively charged cautions and negatively charged anions. When an electrical potential is then passed through the solution. The cautions migrate to the negative electrode and the anions to the positive electrode. Semi permeable membranes are commercially available that allows the passage of only one charge. Caution exchange membranes are permeable only to positive ions and a anion exchange membranes are permeable only to negative ions. When a series of these membranes are placed alternatively in a solution, and a voltage applied, the solution between one pair of electrodes becomes clarified as the ions concentrate in the solution in the adjacent compartments.

The other rarely used tetchiness is evaporation and solvent extraction. In the case of evaporation, the rinse liquor is evaporated to give a metal concentration. This makes the concentration suitable for the direct reuse in the plating bath. Cd^{2+} ion removing by this process is rare and occurs only under special circumstances. On the other hand, the solvent used in extraction operations are too expensive to be used just once, and furthermore the contaminants are highly concentrated in the extract. Therefore, the spent solvent from a liquid-liquid extraction operation needs to be further treated to reclaim the solvent for reuse and to reduce further the volume in which the contaminants is contained.

Hence, most of the above mentioned traditional technologies are based on physical displacement of chemical replacement [8, 9]. These are often ineffective particularly for the removal of heavy metal ions at low concentration [10]. They may require pretreatment and their by products are often hazardous. Treatments, such as ion exchange, electrolysis and reverse osmosis require high capital investments and running cost. To effectively decrease metals to acceptable limits by chemical precipitation treatment requires a large excess of chemicals, which generates volumetric sludge and increases the cost of treatments. (The disposal of toxic sludge generated, adds further burden on the techno – economic feasibility of the treatment processes).

All this limitation of the conventional methods implies so far, lead to the search for an alternative treatment. Efficient and eco friendly technologies are thus needed to be developed to reduce heavy metal content in waste waters at discharge to acceptable level at affordable costs [11].

Biosorption:

The assessment of the metal binding capacity of some types of biomass has gained momentum since 1985 [4]. It was only in the 1990s that a new scientific area developed that could help to recover heavy metals, Biosorption [11]. The pioneer reports described how abundant biological materials could be used to remove, at very low cost, even small amount of toxic heavy metals from industrial effluents. The uptake of heavy metals and radioactive compounds as a result of physiochemical interactions of metal ions with the cellular compounds of biospecies is referred as Biosorption (Kapoor and Viraraghavan, 1998). Recent studies showed that heavy metals can be removed by inexpensive biological materials such as algae, fundi and bacteia (Brierley, 1990; Crist et al., 1994; Fourest and Roux, 1994). The Biosorption mechanisms can be in general metabolism independent or related to the microbial cell activity (i.e., metabolism dependent). Researches on Biosorption reveal that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through different sorption process of ion exchange, complexation, chelation, microprecipitaiton, etc.

Availability is a major factor to be taken into account to select biomass for clean up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. Sea weeds, molds, yeasts, bacteria, crab shells, among other kind of biomass, have been tested for metal Biosorption will very encouraging results [11]. Maintaining a viable biomass during metal adsorption is difficult because it requires to the micro organisms (Spinti et al., 1995). Use of dead microbial biomass as well as biowastes can avoid this problem. The biowastes can be regenerated and reused for May cycles. Some of the biowastes reported to effectively sequester heavy metals include waste tea, exhausted coffee, walnut skin, rice husk, wheat bran, black gram husk and coconut fiber for Cd [12, 13, 22]: polymerized rice bran, rice hulls, soybean hulls and cotton seed hulls for Cu, Ni and Zn [16, 17]. A whole new family of suitably formulated biosorbent can be used in the process of metal removal and detoxification of industrial metal bearing effluents. Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals [18, 19]. When choosing the biomass for metal Biosorption experiments, its origin is a major factor to be taken into account.

Biomass can come from:

1. Industrial and agriculture wasters which should be obtained free of charge;
2. Organisms easily available in large amounts in nature
3. Organisms of quick growth, especially cultivator propagated for Biosorption purpose.

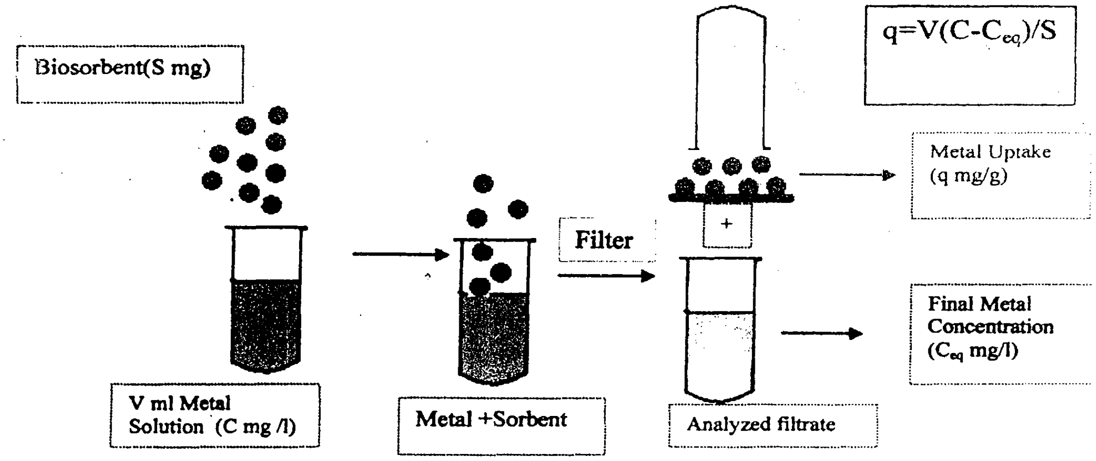
Cost effectiveness is the main attraction of metal Biosorption [12]. Recovery of the deposited metals from the saturated biosorbents can be accomplished because they can often be easily released from the biosorbent in a concentrated washed solution which also regenerates the biosorbent for subsequent multiple reuse. This phenomenon is named as desorption of the metal ions.

The Biosorption of a certain metal by a certain biomass depends on factors such as:

1. The number of sites in the biosorbent material
2. The accessibility of the sites
3. The chemical state of the site (i.e. availability)
4. Affinity between site and metal (i.e., binding strength) [12].

The extent to which the site can be used by a given metal depends on its binding strength and concentration as compared to the metal already occupying the site. Metal sorption performance depend on some external factors such as pH, other ions in solution, which may be in completion, organic material such as complexing agents, cell metabolism products, which may cause metal precipitation and temperature.. Experimental sorption data are in general representing by different empirical or physical models, where heavy metal adsorption is described by mathematical relationships between the concentration of the heavy metal in the liquid phase, in equilibrium condition at constant temperature and pH. The quantitative foundation for comparing any sorption process is the relatively simple batch equilibrium contact experiment [21, 22]. It allows enough time for establishing equilibrium between the metal immobilized, sequestered in the solid material (sorbent) and the metal still left in the solution [12]. Meaningful comparison of Biosorption performance may be done on an equal basis best provided by comparing the solvent metal uptake (q) at the same equilibrium, and the final, residual metal concentration (C_{cq}). The equilibrium (metal) uptake and concentration relationship is expressed by the convention sorption isotherm curves.

These isotherms have been widely applied science they are simple, give a good description of experimental behavior in a large range of operating conditions and are characterized by a limited number of adjustable parameters.



A brief account on Lathyrus Sativus:

Lathyrus Sativus (grass pea or chickling vetch, guaya in Ethiopia, Kheshari, in India; IPBO, 2003) is a common food legume widely grown and eaten in many parts of the world (Jaby El-Haramein et. al, 1988). This annual herb belongs to the family Leguminosae (Papilionoideae).

The nutritional status of L. Sativus is similar to that of other food grain legumes like Pisum sativum. Vicia faba, etc. (White et. al. 2001). Its seeds are rich in protein by 20-32%.

However, L. Sativus can contain a large number of antinutritional substances that can reduce their potential as a raw, unprocessed foodstuff (Foster et al. 1996. Grela et al 2001). Most notable is a neurotoxin. 3-N-Oxalyl-1-2-3 diaminopropionic acid (acronyms: - oxalyl-diamino-propionic acid or ODAP and –oxalyl-amino-alanine or BOAA), which can cause a paralysis of the lower limbs knows as 'lathyrism' (Hanbury et al., 2000; Grela et al, 2001). Other antinutritional substances are protease and amylase inhibitors, lectins, tannins, alkaloids, vicine, convicine, non starch polysaccharides etc.

Most poor people of the developing countries like India, Bangladesh, etc the grain as complementary or sole source of calories and proteins. The 'dal' obtained from grains are the most common item sold in the retail markets. The flour made by grinding either whole or split seeds is also sold in the market.

During the processing of grains, the seed coat or husk of Lathyrus Sativus is obtained as a waste of no economic value. The present work concentrates in investigating the potential of this priceless agro waste as an efficient biosarober of Cd^{2+} ions from low concentration metal solutions.

Aims and Objectives:

The aim of the present investigation is to assess the ability of the husk of Lathyrus Sativus to adsorb Cd^{2+} ions from aqueous solution containing cadmium and also to study the kinetics of adsorption process. In continuing the search for diverse and more efficient heavy metal biosorbers, a wide variety of biosorbents have been tested in the present occasion which include dried powder of jute stick, jack fruit seeds dust, Bagassee pith, water hyacinth, and also Saccharomices cerevisa and Mucor rouxii. Among the sorbents tested husk of L. Sativus, an agro waste having no commercial value and been found to be best to remove Cd^{2+} ions form waste water.

In particular the following aspects have been taken in consideration:

- Selection and processing of the biosorbent material
- Optimization of procedures and conditions including physiochemical parameters (i.e., influence of pH, temperature, biomass and metal concentrations on the Biosorption phenomenon).

- Kinetics study for the determination of the rate of metal (Cd^{2+}) biosorpt8on and determination of the contact period of the experiment.
- Recovery of the deposited Cd^{2+} ions from saturated biosorbents by different desorbing reagents at detected pH, to study their effectiveness.
- Study of the kinetics of Cd^{2+} ions desorption using the most suitable desorbing agent.

Materials and Methods:

Materials:

All the chemicals and reagents used in this experiment were purchased form Merck, Germany.

Biomass:

The seed coat of Lathyrus Sativus was collected from local farmhouse, where it is generated as a primary waste. The husk pieces were extensively washed in running tap water for 2 -3 hours to remove dirt and other particular matters followed by washing with distilled water, repeatedly till clear of any coloration. The washed husk was dried at 80°C for 24 hours; powdered by blending and stored in desiccators until use.

Metal solution:

Desired concentrations of cadmium solution were prepared by diluting standard Cd^{2+} stock solution [$Cd(SO_4)_2$, $8/3H_2O$] of concentration 250 +/- 2mg/1. pH of the solution was adjusted to 5.0 and the temperature was maintained at 20°C, unless otherwise stated, 0.1 (M) HCI or 0.1 (M) NaOH was used to adjust the pH. Fresh dilutions were used for each Biosorption study.

Biosorption procedure:

The Biosorption capacity of Lathyrus Sativus husk was determined by contacting various concentrations (5-250 mg/1) of 50ml Cd solution in 250ml Erlenmeyer flasks, with 0.5g of husk. The mixture was shaken on an automatic shaker at 150 rpm in tightly stoppered flasks for 60 minutes at 20 +/- 1°C followed by filtration through glass wool to separate the biomass from metal solution. Residual concentration of Cd^{2+} in the filtrate was determined using atomic absorption spectrophotometer (Perkin Elmer Model No. 2380).

The Biosorption phenomenon of any metal is critically linked with pH [24]. Not only different metals now different pH optima for their sorption but may also vary from one kind of biomass to other [25, 26, 27]. In order to investigate the effect of pH on Cd sorption by the husk of Lathyrus Sativus, a series of cadmium solutions of different concentrations (ranging from 5-250 ppm) in a fixed volume (50 ml each) were equilibrated at different pH values of 3, 4, 5 and 6. A fixed mass (0.5g) of husk was added in each and shaken in a rotary shaker for 60 minutes as described above. After filtering through glass wool, the Cd^{2+} ion concentration in the filtrate was determined by AAS.

The effect of temperature on the phenomenon of Biosorption was also determined similarly by equilibrating the sorption mixture at different temperature of 10°C, 20°C, 30°C and 40°C. During the study of temperature effect, pH of the solutions, along with biomass and metal concentrations were kept unaltered. Husk free blanks were used as control.

For the determination of the rate of cadmium Biosorption by L. Sativus husk, 50ml Cd solution (20 ppm, pH 5.00 was taken in 250ml Erlenmeyer flask. To it 0.5g husk was added and incubated at 20 IoC under shaking condition (120 rpm). At different time intervals an aliquot was taken, filtered through glass wool and metal concentration of the supernatant was measured.

Desorption procedure:

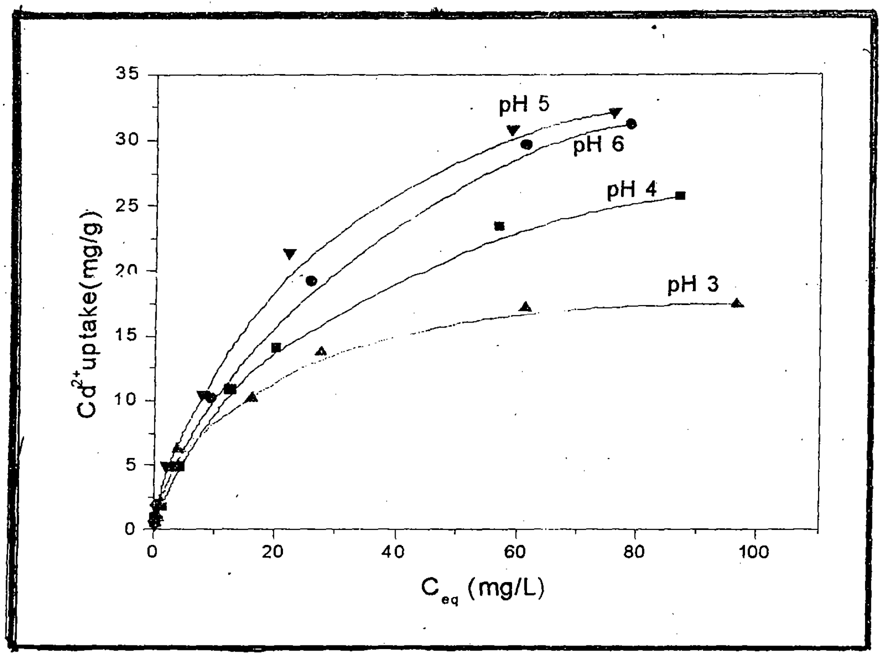
Various types of desorbing reagents, viz, mineral acids (HCI, HNO_3, and H_2SO_4) and aqueous solution of ethylene diamine tetra acetic acid (EDTA) disodium salt were tested at pH1 and pH2 (0.1M and 0.2M in case of EDTA), to recover Cd^{2+} from loaded biomass, 0.5g husk was contacted with 50ml of 20mg/1 Cd solution for 60 minutes at 20 +/ 1°C and pH=5.0 under shaking condition, and separated by filtration. The cadmium loaded biomass was then transferred to 10ml and 20ml of various desorbing agents, and shaken on a rotary shaker at 100 rpm for 30 minutes at 20 +/- 1°C. The biomass was removed by filtration and cadmium content in the filtrate was determined.

In order to study the desorption kinetics, the most suitable desorbing agent (as determined from the previous experiment) was used and the procedure was conducted at respective contact periods ranging form 30 seconds to 2 hours. The physio chemical parameters of the experiment, such as pH, temperature, metal and biomass concentrations were kept fixed as before. After the sorption was over, the metal loaded husk was added into the selected desorbing reagent for respective time periods. Then the husk was separated by filtration and cadmium content of the filtrate was measured by AAS.

Results and Discussion:

1. Effect of pH:

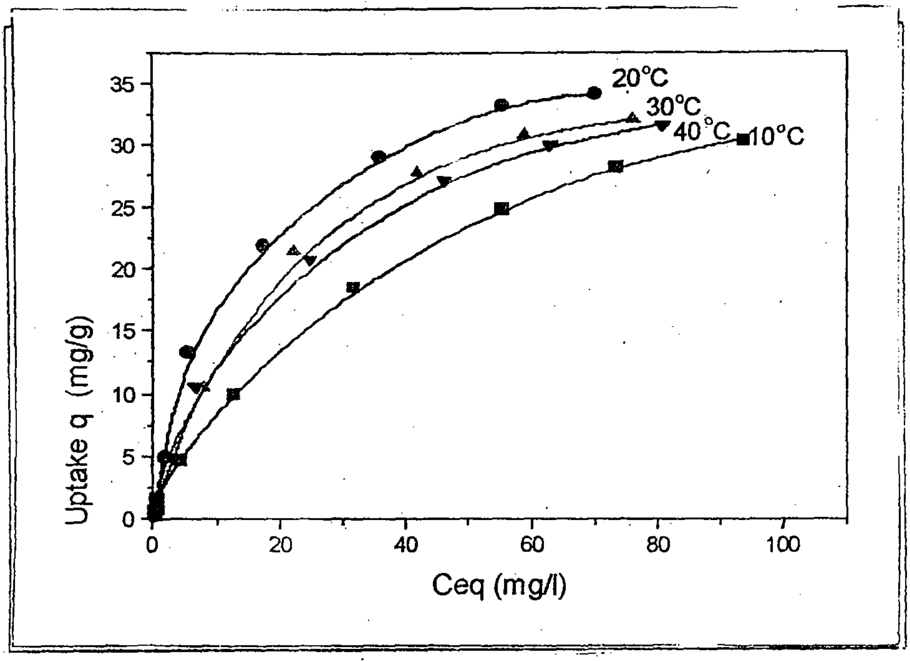
Effect of pH on of Cd^{2+} ions by Lathyrus Sativus husk has been presented in Fig. 3. It appears from the figure that adsorption increases with the increase in pH. Availability of negatively charged groups at the biosorbent surface is necessary for the sorption of metal to proceed [28]. At pH 3.0, such sites were expected to be available at a very low amount due to competition between Cd^{2+} ions and H+ and H_3O^+ ions at such a low pH. Optimum cadmium removal occurred at pH 5.0.



From the earlier records, maximum uptake of Cd, Cr, Cu, Fe, Ni, Pb and Zn by seaweed biomass has been reported to occur with the pH optimal range of 5-6 [29]. In the present occasion further increase in pH form 5.0-6.0 resulted in slightly lower biosorption. This may be attributed to the decreased solubility of metals at high pH [24]. Hence, it can be concluded from the observation that pH 5.0 is the optimum pH for biosorption of Cd^{2+} ions by Lathyrus sativus husk.

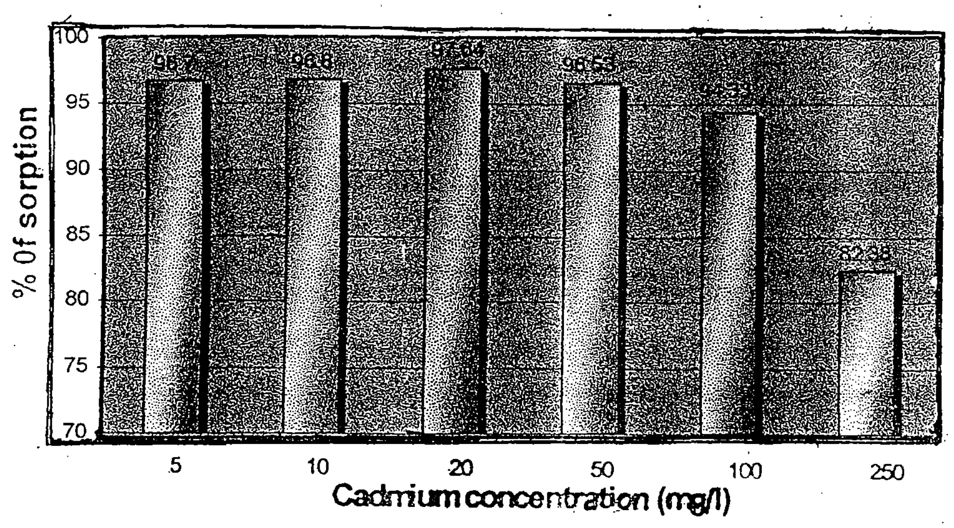
2. *Effect of temperature:*

Temperature is another most significant physico chemical parameter of any experimental set up. So, optimization of temperature for a specific study is an important aspect. The sorption of Cd^{2+} ions by Lathyrus sativus husk was investigated at four different setups: 10°C, 20°C, 30°C and 40°C (as shown in Fig. 2). Sorption was observed to enhance as the temperature was increased from 10°C to 20°C. Maximum uptake of cadmium has been found to occur at 20°C. Biosorption at 20°C was slightly more than at 30°C. Further increase in temperature to 40°C resulted in lower biosorption. Thus, from the study 20°C was marked to be the optimum temperature for biosorption of Cd^{2+} ions by L. sativus husk.



3. ***Effect of metal concentration in biosorption***:

Effect of Cd^{2+} ion concentration on biosorption was determined by contacting different concentrations of cadmium solutions (5, 10, 20, 50, 100, 250 ppm) in same volume (50 ml) with same amount of husk (0.5g) added to each at the optimized pH 5.0 and temperature 20°C. The percentage of adsorption was observed to increase with the increase in metal concentration. The maximum uptake occurred in 20 ppm Cd^{2+} solution. Further enhancing the concentration (to 50, 100 and 250 ppm) the biosorption was not found to increase any more as the binding sites of 0.5g husk has already became saturated. The observation indicates that the optimum concentration to be absorbed by 0.5g Lathyrus sativus husk is 20 ppm at 20°C and pH 5.0.



4. Adsorption isotherm studies:

The adsorption data follows two classical isotherm theories, which are Langmuir isotherm and Freundlich isotherm, respectively. Mathematical representations of these two isotherms are as follows:

- Langmuir isotherm:

$$Q_e=(K_LC_{eq})/1+a_LC_{eq})$$

A linear expression of Langmuir equation is:

$$C_{eq}/Q_e=1/K_L+(a_L/K_L)\ C_{eq}$$

- Freundlich isotheri:

$$Q_e=K_FC_{eq}1^{-n}$$

A linear expression of freundlich equaiton is:

$$Logq_e=logK_F+(1/n)logCeq$$

Where:

C_{eq} = equilibrium metal ion (Cd^{2+}) concentration (mg/L).

Q_e = amount of metal ion (Cd^{2+}) biosorbed per gram of husk (mg/g).

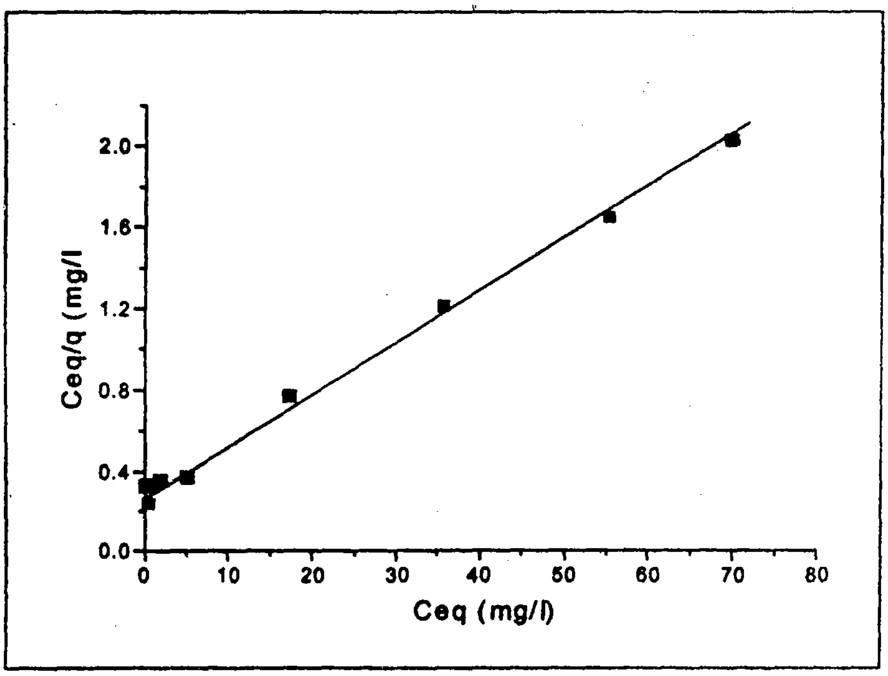
K_L, a_L = Langmuir isotherm constants

K_F = freundlish isotherm constant

1/n = heterogeneity factor.

If the adsorption data follows Langmuir isotherm than a linear relationship would be obtained between C_{eq}/Q_e and C_{eq} from which the two constants, K_L and a_L can be calculated. Alternatively, if the adsorption data follows the Freundlish isotherm then $\log q_e$ would give a straight line from which K_F and 1/n values can be calculated from intercept and slope respectively.

In the presence experiment, for adsorption isotherm study, 20mg/L of Cd^{2+} ion solution was biosorption by 0.5g husk at 20°C and pH 5.0. The data on conversion to both the Langmuir adsorption isotherm model and Freundlich isotherm model yielded straight lines. But, by comparing the liner correlation coefficients ®, it was concluded that the Langmuir isotherm (r=0.9980) was more suitable than the Freundlich isotherm (r=0.892). The Langmuir adsorption isotherm assumes that the adsorbed layer is one molecule in thickness and the theoretical monolayer saturation capacity. K_L/A_L gives Q_o. The isotherm constants a_L, K_L and the equilibrium monolayer capacity Q_o are 3.731 (dm^3/g), 4.322 (dm^3/mg) and 0.861 (mg.g) respectively.



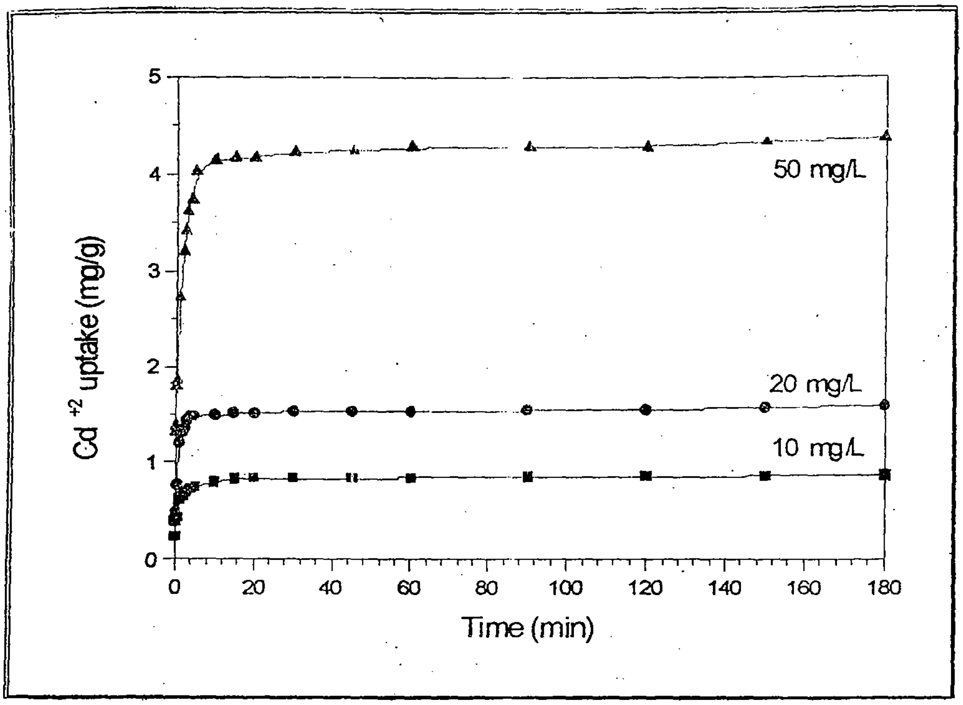
The plot in Figure 6 is typical and demonstrates that the Langmuir equation provides an accurate description of the experimental data, which is further confirmed by the extremely high value of correlation coefficient.

5. Kinetics study for the determination of the rate of cadmium biosorption by L. sativus husk:

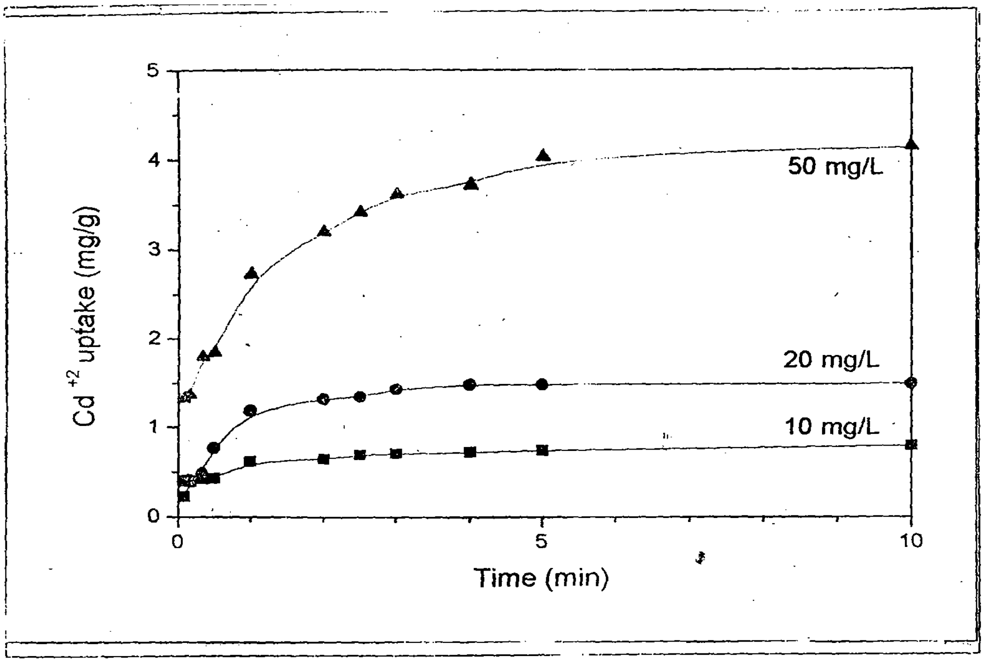
As the sorption percentage of 10mg/1, 20mg/1 and 50mg/1 metal solution were found in close proximity (Fig. 5), all of these concentrations were studied separately to determine the kinetics of the sorption process.

It was found that most of the metal uptake (more than 95%) by L. sativus husk occurred within the first 15 minutes, in 11 the three cases.

Hence, the sorption of cadmium by the husk biomass was very rapid, regardless of the metal concentration (Fig 7).

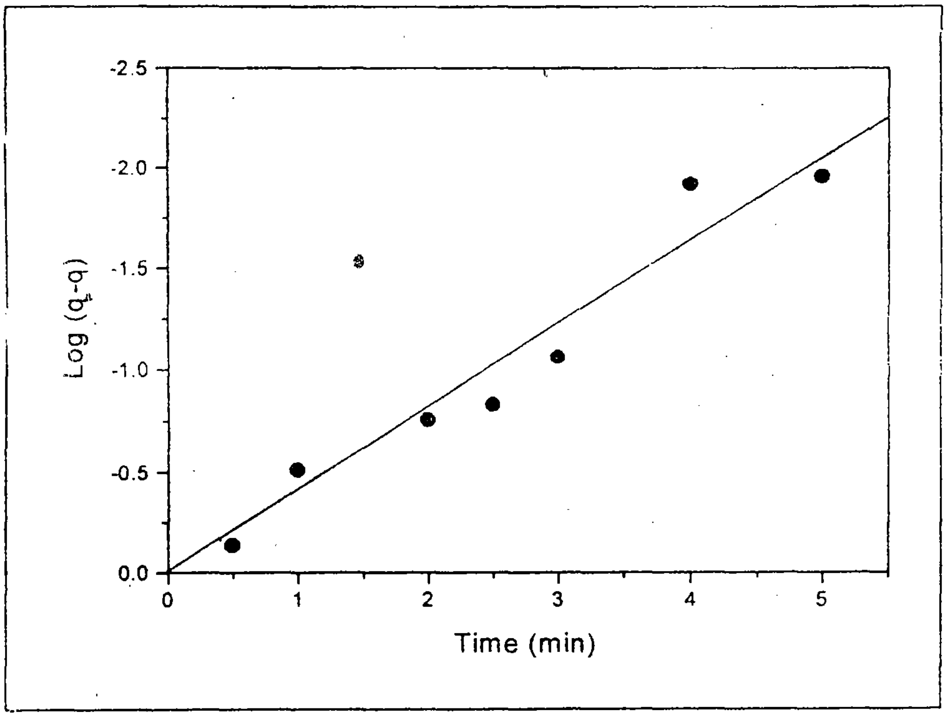


The sorption equilibrium was also achieved within the short period of 10 minutes (fig



It is evident from the above figures that initially the sorption percentage increased regular with time up to 10 minutes and then there was no more increase in the sorption percentage. It can be explained on the basis of the availability of greater binding sites during the initial rapid phase of biosorption. As the binding sites became saturated within the first 10 minutes, no further sorption can take place. That is why the graph maintains constancy beyond 10 minutes.

The rate of the reaction was found to follow the first order kinetics as it satisfied the Lagergren's equation and gave a straight line (where, r=0.983) when log (Q_eq) was plotted against time (1), where Q_e cadmium uptake / g at equilibrium(mg/g) and q is cadmium uptake/g at different contact times (mg/g).



6. *Desorption of cadmium:*

Regeneration of cadmium from Cd-laden L. sativus husk biomass by the procedure of Desorption was investigated for several desorbing agents. The percentage of desorbtion of Cd^{2+} ions by three mineral acids (HC_I, H_2SO_4 and HNO_3) and disodium salt of EDTA are represented in Table 1.

Table 1.

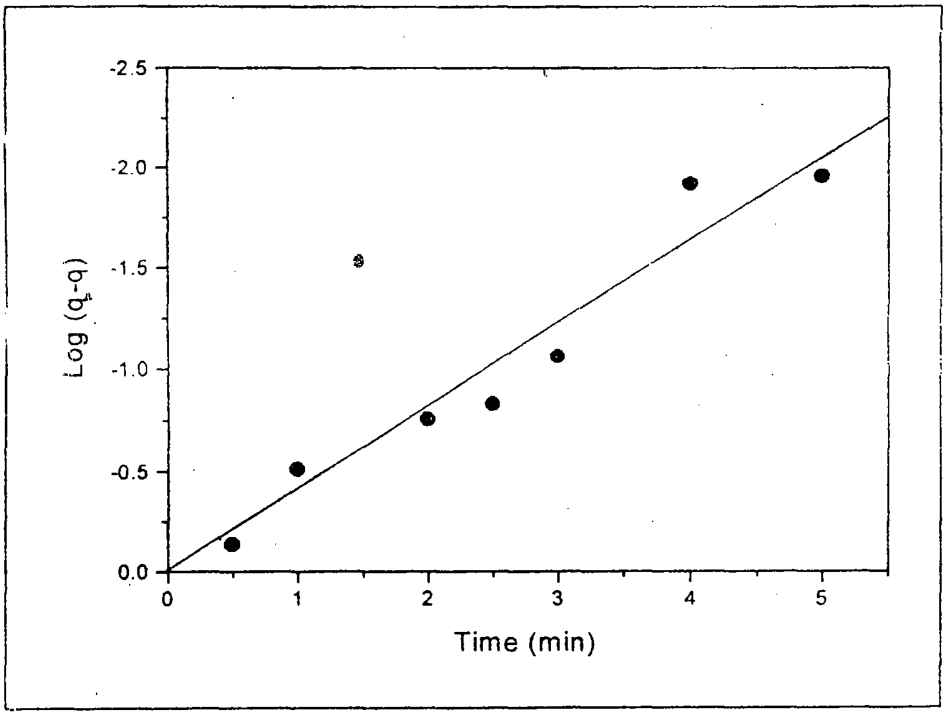
Recovery of cadmium from husk biomass by various desorbing agents.

Desorption agents	**Amount of Cd2+ Recovered (%) (in 10ml desorbing Agents)**	**Amount of Cd2+ recovered (%) (in 20ml desorbing agents)**
HCI (pH 1)	97.14	97.35
HCI (pH 2)	22.33	27.25
H_2SO_4 (pH 1)	96.04	96.17
H_2SO_4 (pH 2)	35.71	36.13
HNO_3 (pH 1)	93.36	94.59
HNO_3 (pH 2)	38.02	39.64
EDTA (0.1 M)	90.63	91.78
EDTA (0.2 M)	93.71	95.29

Conditions: husk biomass 0.5; Cd solution 50ml, 20mg/1; sorption ~ 98%; Desorption time: 30 minutes.

Since, among the investigated desorbing reagents HCI (pH 1) showed comparatively better cadmium Desorption activity and being cheaper, it was selected as the most suitable desorbing agent.

As the Desorption percentage in 10ml and 20ml HCI (pH 1) are almost equal, i.e., 97.14% and 97.35% respectively, 10ml HCI (pH 1) was used in studying the Desorption kinetics.



It is evident from the graph that the maximum amounts of cadmium Desorption occurred within 60 minutes. Thereafter, there was no increase in Desorption amount as the system has reached its equilibrium.

Conclusion:

The following conclusions can be drawn from the present investigation:

- About 98% of cadmium can be removed from low concentration waste water by the husk of Lathyrus sativus.

- 1g of the biosorbent can remove 20.8mg of Cd (II) from waste water
- The optimum pH and temperature of the biosorption process are 5.0 and 20°C respectively
- The sorption equilibrium was attained within a short period of 10 minutes
- Equilibrium isotherm follows Langmuir isotherm model
- HCI (pH 1) can desorb more than 97% of cadmium form the Ce-laden biomass within 1 hour. Desorbed husk can be reused.

Hence, it is evident that the procedure of biosorption is a rapid, economic as well as eco friendly process, where both the metal and the sorbent can be recovered at the end, in order to reuse again.

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DR ACHINTO PARAMANIK

I have completed my **M.SC, PH.D,** in Microbiology.I have a teaching & research experience of 05 years,teaching subjects: Biotechnology,Biology,Cell biology, Plant/Animal Biotechnology, Cell biology & Genetics etc.I Investigated around 08 research problems in various fields of science including Molecular biology, Molecular marker studies in anticancer plants, horticulture plants, ornamental plants and other crop plants. I have received 05 awards as young scientist, etc with 20 no. of publications international/national, with 35 no. of conferences international/national . I am working as a Lecturer in Biology,ALFALAH GROUP OF INSTITUTIONS,RAMANAGARA DIST,MAGADI-562120,BANGALORE RURAL.I will contribute my research work to my country focusing on assiduous study, teaching learning and research, science and technological innovation. I will contribute to a number of international high-end academic platforms.