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Effect of fungal pretreatment on *Solanum nigrum* L. leaves biomass aimed at the bioadsorption of heavy metals

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Environmental degradation due to water pollution, air pollution and soil pollution has a detrimental effect and create ecological imbalance. These factors are also responsible for the large scale introduction of heavy metal ions into the environment. Conventional treatment methods for the removal of heavy metal ions are not so advantageous as these are highly expensive and also produces toxicant. Keeping this view in the mind, the present study was framed to determine the biosorption of heavy metal ions using *Solanum nigrum* L. leaf biomass alone and also with *Aspergillus oryzae* treated *S. nigrum* leaf biomass. *Solanum nigrum* L. leaf biomass was used as biosorbent for the biosorption of cobalt, copper, iron,lead and zinc.Unaided *S. nigrum* leaf biomass was the better absorbant of lead (Pb) whereas when inoculated with *A. oryzae* cobalt (Co) was more biosorbed than any other heavy metal. Overall, the results of the present study propose that the traditional plant *S. nigrum* could be used for the bioadsorption of heavy metals.

Keywords: Biosorbent, Biosorption, Conventional methods, Environmental pollution, Heavy metal ions, *Solanum nigrum* L.

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Indian economy largely depends on the agriculture sector, and it is the primary source of livelihood provider for the population of India for so many years. At the time of independence, there was a slow growth rate of the industrial sector and GDP (Gross Domestic Product) after that rapid increase was observed in both[1]. This increasing growth of the industrial sector was directly linked with the degradation of the environment and its natural resources, crop productivity, and human and animal health[2]. Anthropogenic activities and overexploitation of natural resources by the increasing rate of industrialization and continuous urbanization are mainly responsible for the contamination and pollution of the aquatic system[3]. Heavy metals are naturally occurring elements that have a high atomic weight and a density compared to water[4]. Pollution and contamination of wastewater due to the presence of heavy metals now becoming a serious environmental problem globally because of large scale discharge of wastewater containing heavy metal ions by the various industries[5]. Water bodies now continuously becoming polluted due to the persistent and non-biodegradable nature of metal ions which tend to bioaccumulate, a condition in which the concentration of a chemical increases over time[6].

Current wastewater treatment systems are highly expensive, and due to the lack of financial ability, such type of industries tend to discharge their effluents directly into the water bodies in a non-ecofriendly manner[7]. Precipitation, ion exchange and electrolytic techniques are the conventional treatment methods for the elimination and detoxification of metal ions from the industrial effluents[8]. Chemical precipitation of metal ions is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers[9]. However, due to the production of a large number of toxic compounds, this method is not quite effective[10]. In the ion-exchange process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin[11]. Whereas, the high cost and partial removal of

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certain ions are the two major disadvantages of this method. In electrodialysis method, heavy metals are removed with the help of ion-selective semi-permeable membrane[12]. However, in this method membranes are clogged due to the formation of metal hydroxides[13].

A new approach of Biosorption based on the metal binding capacities of various biological material developed during the 1990s. Biosorption can be defined as the efficiency of bioadsorbent by which they can accumulate heavy metal ions from wastewater through the metabolically regulated or physical-chemical pathway of uptake[14,15]. In comparison to the conventional methods bioadsorption has several advantages such as (i) the high efficiency, (ii) low cost, (iii) no any additional requirement of nutrient, (iv) regeneration of the bioadsorbent, (v) possibility of recovery of metal, and (vi) reduction of chemical or biological sludge[16]. Biosorption technology is mainly based on surface adsorption. Biosorption defines the removal of heavy metal toxicants, and pollutant from the environmental compartment and this technology represents an alternative to the traditional treatment method for the recovery of metal ions[17]. Biosorption is a physicochemical process in which the biological material can be utilized for removal of toxic metal ion from solution[18]. In comparison to the conventional treatment methods, biosorption reaction kinetics are fairly rapid as the majority of metal ions are removed within a few minutes of reaction which is highly desirable to reduce the daily running costs of the treatment plant[19]. Various studies indicated that the non-living plant biomass has the metal removal capacity and hence can be effectively utilized for the detoxification and elimination of heavy metal ions from the environment[20–23].

The principal driving force for metal ion biosorption is a net negative surface charge of the biomass. Obviously, the higher the biomass electronegativity, the greater must be attraction and adsorption of heavy metal cations[24]. The surface of biomass consisting primarily of proteins, carbohydrates, nucleic acids and lipids, imparts a negative charge due to the ionization of organic groups such as carboxylic, aliphatic, aromatic and amino groups and inorganic groups such as hydroxyl and sulphate groups and these groups can attract and sequester metal ions[25,26]. Bacteria, algae, fungi, yeast, living organisms and dead remains of microorganisms exhibit biosorption potential for the metal ion removal[27]. The inactive dried microbial biomasses can bind with metal ions and can be utilized as an effective method for the recovery of metal ions[28]. The process of surface adsorption of heavy metal ions occurs by the dead microbial biomass and shows higher capability for metal ions removal from aqueous solutions[29].

While choosing any biomass for metal Biosorption, its source is a major factor to be taken into account. It may come from (a) industrial waste which is obtainable free of charge; (b) organisms readily available in large amount in nature, and (c) organisms of quick growth especially cultivated for biosorption purposes. *Solanum nigrum* L. being an abundantly available weed might be exploited for this purpose[14,30]. *Solanum nigrum* L. belongs to the family Solanaceae, commonly called Makoi. It is a short-lived annual or perennial herbaceous plant or small shrub and a common weed[31]. It is native to north-western Africa, Europe, western and central Asia, China, and the Indian Sub-continent (i.e. northern India, Nepal and Pakistan).Moreover, *S. nigrum*, is a traditional remedy for various type of ailments like ulcer, liver toxicity and a cure for pancreatic cancer. These properties of *S. nigurm* plants are due to the presence of phenolic acids. The plant has antiseptic, antidysenteric, emollient, diuretic and laxative properties[32,33].

Copper, lead, cobalt, iron and zinc are amongst the metals which find their way into the water bodies and ultimately persist in the environment. As much as 9.3 kg copper, 489 kg iron, 3.36 kg lead and 37.2 kg zinc per day enter the Yamuna River before it reaches Delhi. The present work, therefore, was focused on the utilisation of *Solanum nigrum* leaves biomass for the biosorption of (a) cobalt (b) copper, (c) lead, (d) iron, and (e) zinc. An attempt was also made to find out the effect of fungal pretreatment on the capacities of *Solanum nigrum* leaves biomass for the biosorption of heavy metal ions.

Materials and Methods

a. *Collection of Solanum nigrum plants and preparation of leaf powder*.

Fifty healthy plants of *Solanum nigrum* were collected from the Meerut, Uttar Pradesh coordinates at (28.9845° N, 77.7064° E) and washed thoroughly with water to remove dust. Now, these plants were dipped in the alcohol for 10 minutes so that the surface microflora is killed. After surface sterilization the plants were shade-dried at room temperature.

After their separation, the leaves were grounded in a grinder for obtaining the fine powder. These powdered leaves samples were stored at a dry place. About 10 g of *Solanum nigrum* leaves powder added into the number of sterilized flasks, and these flasks were then inoculated with *Aspergillus oryzae* spore suspension. After 3-7 days, fungal colonies appeared onto the leaves.

b. *Adsorption of metals by the untreated and Aspergillus oryzae treated Solanum nigrum leaves*.

For this experimental study, five metals, i.e., cobalt (as cobaltous nitrate), copper (as cupric sulphate), iron (as ferrous sulphate), lead (as lead nitrate) and zinc (as zinc chloride) were selected. Stock solutions of different metal salts, i.e., (i)100 ppm of cobalt; (ii) 300 ppm of copper; (iii) 500 ppm of iron; (iv) 500ppm of lead and (v) 250ppm of zinc were prepared

Mahvi *et al*.[34] method with slight modification was adopted to determine the efficiency of biomass varieties under study (a) untreated *Solanum nigrum* leaves, and (b) *Aspergillus oryzae* treated *Solanumnigrum* leaves.

100 ml of 100 ppm solution of cobalt was added into each of nine sterilized flasks. These 9 flasks were then divided into three sets of three flasks each. Now in these sets of flasks, biomass sample were added in the following manner:

1. 1.0g leaf powder of *Solanum nigrum* plant was added into 3 flasks
2. 1.0g leaf powder of *Solanum nigrum*plant containing fungal biomass was added into 3 flasks
3. A set of 3 flasks served as control (nothing was added into these flasks)

The content of these flasks was then filtered through Whatman number 40 filter paper after two hours of contact time. The Merckoquant Metal Analytical Test Strips were used to determine the metal concentration of filtered solutions and to find out that range in which the concentration of metal present in the test filtrate. The volume of the content was the increased upto 300 ml, and thereafter a few drops of HNO_3 (concentrated) were added. Metal solutions were analysed with the help of Atomic absorption spectrophotometry.

A similar methodology was repeated for the analysis of other metallic solutions.

Following method adopted by Hussein *et al.*[35] was used for the calculation of metal bound by the bioadsorbent:

$$Q = V\,(Ci - Cf) / m$$

where,

Q is the mg of metal adsorbed by per g of bioadsorbent,

V is the volume of liquid sample (ml),

Ci is the initial metal ion concentration (mg/l),

Cf is the final metal ion concentration (mg/l),

m is the weight of the used biosorbent on a dry basis (mg).

The biosorptive potential and quantityof particular biomass were exemplified as: very poor (in the range between 0-10), poor (10-20), moderate (20-40), good (40- 60), very good (60-80) and excellent (80-100).

Results and Discussion

In the present experimental study, the potentiality of *Solanum nigrum* leaves (dried biomass) for the adsorption of cobalt, copper, iron, lead and zinc was investigated. For this purpose, uninoculated *Solanum nigrum* leaf powder and inoculated (with *Aspergillus oryzae*) leaf powder was used. Table 1 shows the results obtained by the testing of metal ion solution after the two hours of the bioadsorption. In the present work, preliminary testing of metal ion solution was carried out by the use of Merkoquant metal analytical testing strips so only the range of the amount of metal ions remaining in the solution (after biosorption) are presented in Table 1. The leaves biomass of *Solanum nigrum* is highly effective material for the bioadsorption of all the metals studied

Table 1 — Approximate amount of heavy metal ions remaining in the solution after two hours of bioadsorption with uninoculated *Solanum nigrum* leaf biomass and *Solanum nigrum* leaf biomass inoculated with *Aspergillus oryzae*.

Metal	The concentration of metal in the original solution (ppm)	Amount of metal remaining in the solution after biosorption (ppm)	
		Uninoculated *Solanum nigrum* leaf biomass	*Solanum nigrum* leaf biomass inoculated with *Aspergillus oryzae*
Cobalt (as cobaltous nitrate)	100 ppm	0-10	± 30
Copper (as cupric sulphate)	300 ppm	30-100	± 100
Iron (as ferrous sulphate)	500 ppm	± 100	± 250
Lead (as lead nitrate)	500 ppm	Nil	± 100
Zinc (as zinc chloride)	250 ppm	100-250	± 100

in the present work (Table 1). It was noticed that zinc metal ions were the least adsorbed metal ions, and after the biosorption, the concentration of lead remaining in the solution turned out to be nil. So this study clearly showed that lead metal ions were the most effectively adsorbed metal ions (Table 1). Cobalt was followed by iron, as the concentration of iron was 100 ppm as compared to 500 ppm in the original solution (Table 1). Iron was followed by copper, as the concentration of copper in the remaining solution was less than 30% (Table 1). The inoculation with *Aspergillus oryzae* appears to have an adverse impact on the efficiency of leaf biomass of *Solanum nigrum* plant for bioadsorption of cobalt, copper, lead and iron. It was noticed that for the zinc biosorption, the potentiality of *Solanum nigrum* leaf biomass was positively influenced after colonization with *A. oryzae* (Table 1).

From this work it was concluded that the preferential bioadsorption of metal ions by the *A. oryzae* inoculated *Solanum nigrum* leaf biomass was

Cobalt > Zinc = Copper = Lead > Iron

and the preferential bioadsorption of metal ions by the uninoculated *Solanum nigrum* leaf biomass was

Lead > Cobalt > Iron > Copper > Zinc

Further examination of metal ions containing solutions was carried out through atomic adsorption spectrophotometer, and the results of this examination are presented in Table 2. From this experimental work, it was found that specific uptake of cobalt by uninoculated *Solanum nigrum* leaf biomass was 12.15. Marešová *et al*.[36] observed the utility of moss biosorbent for cobalt metal ion and reported that that the maximum uptake capacity (Qmax) 123 μmol/g for cobalt. Sarwar *et al*.[37] worked on cobalt biosorption by *Trapa natan* biomass and found that the maximum specific uptake for Co (II) was 24.28 mg per gram. Present investigation showed that the specific uptake of copper by uninoculated *Solanum nigrum* leaf biomass was 14.15. Keskinan *etal*.[38] used *Myriophyllum spicatum* for the copper biosorption and found the specific uptake of Cu was 10.37 mg/g. Peng *et al.*[39] studied the biosorption of copper by natural foxtail millet shell and noticed that the maximum biosorption capacities of foxtail millet shell were 11.89 mg/g. The specific uptake of iron by uninoculated *Solanum nigrum* leaf biomass was 26.42**.** Verma *et al*.[40] reported that 5.17 mg/g Fe(II) was adsorbed by *Aspergillus flavus* biomass.

This work indicates that the uninoculated leaf biomass of *Solanum nigrum*was an excellent bioadsorbent for the biosorption of lead metal ions. In the present study, the specific uptake of lead was found to be 51.0. Niu *et al*.[41] reported the specific uptake of lead in the range of 122 by *Penicillium chrysogenum*. Keakinan *et al.*[38] examined the capability of a submerged aquatic plant, *Myriophyllum spicatum* for the biosorption of lead and reported that the biomass of this plant adsorbed 46.69 mg/g lead. Aneja *et al.*[42] observed that the maximum adsorption per unit mass of *Spirulina* (mg g^{-1}), for Pb^{2+} was calculated to be 48.21 mg g^{-1}. Biosorption of lead also reported by the pretreated fungal biomass[43], *Aspergillus terreus*[44] and *Aspergillus versicolor*[45]. Garcia *et al*.[46] found that the two strains of *Bacillus* sp.(C13 and C16) could be used for the lead biosorption. Mahish *et al*.[47] observed the efficiency of *Penicillium oxalicum* biomass for lead biosorption and also reported 89.82% lead adsorption by *P. oxallicum* biomass. In the present experimental work, the specific uptake of zinc metal ions by uninoculated *Solanum nigrum* leaf biomass was 18.10. Sekhar *et al.*[48] reported that the removal of zinc ions increased in the presence of cobalt ions. It may be due to the chemical interaction that occurred between the metal ions and also their interactions with the biomass[49,50]. Mali *et al*.[51] suggested that *Aspergillus flavus* (biomass) could be efficiently used as an effective biosorbent for the zinc metal ions.

Table 2 — Specific uptake of metal ions (Q value) of uninoculated and inoculated Solanum nigrum leaf biomass for all the five metals studied.

Metal	Initial concentration of metal ions (mg/lt)	Final concentration of metal ions (mg/lt)		Specific uptake of metal ions (Q value)	
		Uninoculated *Solanum nigrum* leaf biomass	*Solanum nigrum* leaf biomass inoculated with *Aspergillus oryzae*	Uninoculated *Solanum nigrum* leaf biomass	*Solanum nigrum* leaf biomass inoculated with *Aspergillus oryzae*
Cobalt	100	86.87	84.82	12.15	6.43
Copper	300	156.4	224.50	14.12	7.42
Iron	500	214.50	252.10	26.42	24.53
Lead	500	Nil	92.239	51.0	40.52
Zinc	250	198.95	186.35	18.10	12.26

Nasim and Dhir[52] reported that the production of bioactive compounds or metabolites increased in the medicinal plant on the exposure of heavy metal stress but the exact mechanism of this increase still not clear. Due to the effect of heavy metal ions, there is ROS (reactive oxygen species) generation which triggers the signalling pathway for the production of plant metabolites. Ganeshamurthy[53] reported that heavy metal polluted water of rivers near the urban areas or cities are used by the farmers for irrigation purpose as a result of which these heavy metals get accumulated in the crops (vegetables and fruits). There is an urgent need to monitor the presence of metal contaminants in vegetables and fruits coming from these sources, and, hence, special attention should be focused in this respect. The population of economically weaker sections of developed but mostly developing countries directly depends on the natural resources (fuel wood, fodder, forest, and water). The poverty rate and reduced life quality significantly increased as a consequence of growing nonequivalence competition for the natural resources among the population of economically weaker section in the deficiency of alternate or apparent natural resources[54]. For the regular monitoring of heavy metal pollutant and industrial pollution, various agencies are workable at the national and international platform as well. Ali *et al.*[55] reported that for the monitoring or detection of heavy metal environmental pollutants, different environmental markers (plant and animal species) can serve as bioindicators[56].

Conclusion

This experimental study indicated that *Solanum nigrum* L. leaf biomass is better bio adsorbent. Therefore, various biological materials have shown their potentiality for the adsorption of metal contaminants and hence, can be utilized as biosorbent. Biosorption describes the detoxification and removal of contaminants and heavy metal pollutantsfrom the environmental components. This technology of biosorption represent a much better alternative as it is an inexpensive, time-saving, eco-friendly approach, and it makes convenient reuse of the industrial and agricultural residues.

Conflict of Interest

Authors declare there is no conflict of interest.

Author Contributions

JC, RKJ and PK conceived of and designed the project; supervised the study and corrected the final draft. JC, IS, and AT, performed the experiments and analyzed the data. VKY, IS and PK wrote the manuscript. All authors read and approved the final manuscript.

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