Utilization Certificate

1. **Title of the project:** Biosorption: A sustainable approach in remediation of toxic heavy metals from industrial wastewater/ synthetically prepared solution using snail shell dust and fish scales
2. **Sanction Order no.** RA/231/8103- 8109 dated 8/3/2023
3. **Name of the PI:** Dr. Arti Dogra
4. **Department:** Department of Zoology, University of Jammu
5. **Total Project Cost:** Rs. 2,00,000
6. **Statement of Expenditure:**

Head	**Total grant released**	Total expenditure incurred (INR)	**Unspent Balance (INR)**
Hiring of Services/Honorarium for experts	5000/-	5000/-	0
Purchase of Minor Equipment	85,000/-	84,497/-	503/-
Consumables/Chemicals/ Glassware	75,000/-	74,797/-	203/-
Contingency	20,000/-	20,000/-	0
Field work	15,000/-	14,994/-	6/-
Total	2,00,000/-	1,99,288/-	712/-

Certify that out of Rs. 2,00,000 (Two Lakhs only) of Grant-in-aid, sanction vide order no. RA/231/8103-88109 dated 8/3/2023 in favour of Dr. Arti Dogra (PI), a sum of Rs. 1,99,288/- has been utilized for the purpose of research for which it was sanctioned and the balance of Rs. 712/- remained unutilized.

Signature

PI

Deputy Registrar (Grants)
University of Jammu

Signature

Deputy Registrar

Joint Registrar (Finance)
University of Jammu

Signature

Joint Registrar

Biosorption: A sustainable approach in remediation of toxic heavy metals from industrial wastewater/ synthetically prepared solution using snail shell dust and fish scales

Increased population and high global demand for water have put long-term stress on the world's natural resources. An increase in industries as well as urbanization has resulted in the discharge of untreated wastewater into the aquatic bodies, thus worsening water quality, disturbance of ecosystem balance, and surface and groundwater pollution and contamination. One of the major challenges of the current world scenario is to have access to safe drinking water but this has become out of the reach. In India, about 70% of the total surface water is polluted and the main culprit behind this is untreated discharge from various industries, domestic sewage and agricultural runoff.

It has been estimated that exponential growth of industries contributes about 26% of total water pollution. The discharge from these industries mainly consists of non-biodegradable heavy metals which are highly toxic for all living organisms.

Elements in the periodic table having specific density of about 5 gm/cm^3 and whose atomic weight lies in between 63.5 to 200.6 Dalton are heavy metals. These metals are commonly discharged through various industries (paint, brass, batteries, oil refineries, metal plating units, mineral processing units, chemical, fertiliser and pesticides), mining activities and from automobile emissions. Every year 0.39 million tons of metals are added into environment by various industries, 1.4 million tons each year is contributed by agricultural activities while waste disposal accounts for 0.72 million tons. Heavy metals are considered harmful because of their toxicity, tendency to persist in nature and their ability to bioaccumulate in higher organisms along the food chain through the process of biomagnification. These accumulations could result into life-threatening damages to the biota.

Generally, at low concentration these metals are nontoxic and elements like Zn, Cu, Fe, Mn, Mo, V, Ni and Co act as cofactors for many enzymatic reactions but when their concentration increases beyond the threshold level, they become toxic for the individuals. However, some heavy metals are not at all required by living beings due to their toxic nature even at low concentration viz. As, Cd, Hg, Se, Pb etc.

Keeping in view the detrimental effects caused by these contaminants (heavy metals, dyes and other pollutants) there is an urgent need for their removal from our aquatic ecosystems. To overcome this problem many conventional methods such as reverse osmosis, chemical precipitation, chemical oxidation, membrane separation, ion exchange, filtration, electrochemical treatment, electrodialysis, coagulation or flocculation are employed for removal of these contaminants. Although all these conventional methods are rapid and easy to operate but these advantages are overshadowed by few drawbacks like:

- Very costly to operate and maintain
- Produce secondary pollutants
- Generate toxic sludge which is difficult to dispose
- Requirement of high energy
- Inefficient in removal of heavy metals at low concentration

A very promising technique used nowadays is bisorption/ adsorption for the eradication of pollutants. This technique comprises of two components i.e., adsorbent and adsorbate. Adsorbent is any solid material that is utilized to adsorb pollutants whereas adsorbate is the liquid phase containing contaminants. Biosorption involves the binding of an adsorbate on the surface of any solid surface, i.e., biosorbent because of the high affinity for each other. The mechanisms involved in the adsorption process are either electrostatic attraction, ion exchange or surface complexation. Functional groups such as $-NH_2^-$, -CH, $-PO_4^{3-}$, -OH, C=O, and COO^- are involved during the process of adsorption to form bonds on the surface of the adsorbent.

Although various low-cost adsorbents such as agricultural waste are used as a biosorbents, very little evidence is available regarding the utilization of animal waste. All around the globe, approximately 6 to 8 million tonnes of animal food waste is produced annually. This waste accounts for a nuisance in the ecosystem due to its long persistence in nature and prolonged decomposition time. Animal waste like molluscan shells, crab and prawn carapaces, fish scales, animal bones, horns, eggshells etc., have the potential to be used as a bio-remediator due to their chemical combination and structural properties. The present study thus mainly focuses on the utilization of fish scales and snail shells in the remediation of heavy metal ions.

During the study period, *Bellamya bengalensis* snail shell dust and fish scales were evaluated for the removal of some metal ions like Cadmium, Lead, Nickel etc. from the synthetically prepared solution. To carry out the above experiments following steps were undertaken:

1. Collection of adsorbent

Snail shell

The collection of snail shells were done from the local freshwater bodies i.e., Gho Manasa, Kurian wetland etc. Snail shells were collected by using metallic sieves of pore size 1 mm or directly by hand picking. These were brought to the laboratory and were washed with hot boiling water to remove the visceral mass using forceps. The empty shells were further washed with double distilled water 3-4 times to remove any debris/algae attached and then sun dried for at least 2 days. Thereafter they were kept in the oven at temperature of 105.5 ℃ for 24 hrs and then crushed into fine particles using mortar and pestle/ grinder. The powder thus obtained was passed through different micron sized sieve so as to obtain a homogeneous mixture of snail shell powder. This final powder was then utilized as an adsorbent for further studies (Fig. 1a-d).

Fig.1 (a) Collection site; (b) collection of snail shells; (c) *Bellamya bengalensis* snail shell; (d) snail shell dust

Fish scales:

The local fish market of Jammu was explored for the collection of mixed fish scales which were discarded as waste. These scales were washed repeatedly with water to remove adhering dust and soluble impurities from their surface. They were allowed to dry in sunlight for 2-5 days (Fig. 2). The scales were then kept in an oven at 70℃ till they became crispy and slightly brownish in color. The dried scales were then ground using mortar and pestle. These ground particles were sieved using 100-micron mesh size to obtain uniform adsorbent size. The resulting fish scale powder was stored in air tight container till further use.



Fig.1 (a) Fish scales (b) Fish scale husk (c) Fish scale dust

2. Optimisation of various factors

To determine the optimum conditions for maximum removal of metal ions using snail shell dust, following parameters and conditions were evaluated (Table 1).

Table 1. Various factors influencing adsorption of Heavy metals and their conditions (snail shell dust)

Factors	Conditions
Contact Time (minutes)	10, 20, 40, 60, 80 and 100
Adsorbent dosage (g)	0.2, 0.4, 0.6, 0.8 and 1
Adsorbent size (micron)	100, 200, 300, 400 and 500
pH	2.0, 4.0, 6.0, 8.0 and 10
Initial Concentrations (ppm)	50, 60, 70, 80, 90 and 100

To determine the optimum conditions for maximum removal of metal ions using fish scales dust, following parameters and conditions were evaluated (Table 2).

Table 2. Various factors influencing adsorption of Heavy metals and their conditions (fish scales shell)

Factors	Conditions
Contact Time (minutes)	20, 40, 60, 80, 100, 120,140 and 180
Adsorbent dosage (g)	0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8 and 2
Adsorbent size (micron)	Scales, husk and dust
pH	2.0, 4.0, 6.0, 8.0 and 10
Initial Concentrations (ppm)	50, 60, 70, 80, 90 and 100

3. Adsorption isotherms

To determine the maximum adsorptive capacity of the snail shells and fish scales, mode of binding of heavy metals on the surface of biomaterials, two isotherms were taken into consideration i.e., Langmuir and Freundlich isotherms.

4. Results

The biosorption of different heavy metals from the relevant synthetically prepared aqueous solution was found to be affected by various factors like contact time between the adsorbent (snail shell/ fish scales) and the adsorbate (heavy metal solution), adsorbent dosage, adsorbent size, the pH of the test solution, and the initial concentration of heavy metal. Thus, it becomes imperative to optimise all of these above parameters so that maximum heavy metal removal can be achieved.

For snail shell dust:

- The optimum conditions for maximum removal of 50 ppm Cadmium ions (99.38%) from aqueous solution were 40 minutes of contact time, 0.6 g of adsorbent dosage having 100 micron size at pH 6.
- For Lead, the optimum conditions were found to be 60 minutes of contact time, 0.6 g of adsorbent dosage having 100 micron size, and pH 6 for the maximum removal (99.98%) of Lead from its 50 ppm aqueous solution.
- Maximum percentage removal i.e., 97.24% of Nickel ions from 50 ppm aqueous solution was noticed at 40 minutes equilibrium time with 0.4 g of adsorbent dosage having 100 micron size and pH 6.
- The studies revealed the mode of binding between adsorbent and heavy metals (Cd, Ni, and Pb) to be monolayer as witnessed by the Langmuir isotherm model.
- The adsorption capacity of heavy metal ions was recorded in the order of Nickel (29.14 mg/g) > Cadmium (16.47 mg/g) >Lead ions (16.35 mg/g).

For fish scales dust:

- For Nickel, the optimum conditions were found to be 120 minutes of contact time, 1.2 g of adsorbent dosage of dust-size particles, and pH 6 for the maximum removal (95.81%) of Lead from its 50 ppm aqueous solution.
- For Cadmium, the optimum conditions were found to be 100 minutes of contact time, 1 g of adsorbent dosage having dust-size particles, and pH 7 for the maximum removal (99.9%) of copper from its 50 ppm aqueous solution.
- For Lead, the optimum conditions were found to be 160 minutes of contact time, 1.2 g of adsorbent dosage of dust-size particles, and pH 6 for the maximum removal (97.81%) of Lead from its 50 ppm aqueous solution.

- The studies revealed the mode of binding between adsorbent and heavy metals (Cd, Ni and Pb,) to be monolayer as witnessed by the Langmuir isotherm model.
- The adsorption capacity of heavy metal ions was recorded in the order of Nickel (30.14 mg/g) > Cadmium (28.95 mg/g)> Lead (18.25 mg/g).
- In comparison with snail shell dust, fish scales possess more adsorptive capacity.

All the above results authenticate our project hypothesis and could be utilized for the treatment of wastewater before direct disposal into water bodies. This will also help in the valorisation of animal waste and the reduction of its nuisance in the ecosystem. Moreover, such methods will be the best solution to achieve the sustainability goal.