

Editorial	01
Yellow water Pollution	02
CEPA Programme (SACON)	06
Query Form	08
ENVIS Team	08
Contact Us	08

Editorial

The Salim Ali Centre for Ornithology and Natural History (SACON) brings out the second issue of SAROVAR SAURABH (volume-5), an ENVIS Newsletter on wetland ecosystems, sponsored by the Ministry of Environment and Forests, Government of India. The major goal of the Newsletter is to share information about wetlands with various users and, to highlight conservation issues of relevance to wetland community of professionals, managers, environmentalists and other stakeholders.

This newsletter deals with potential indigenous microbial strains for treatment of industrial and mining effluent in Chromium contaminated waste of Sukinda mines, Orissa and SACON's CEPA activity in Guntur District, Andhra Pradesh. It is again hoped that stakeholders in wetland conservation and all other ENVIS centres can make use of these information effectively to create public awareness for further wetland conservation.

To make this effort worth while, the editorial team of SAROVAR SAURABH seeks active participation of its readers in terms of providing information, news, views, photographs and articles on issues of wetland conservation. To make the newsletter a truly effective forum for all wetland conservation related issues of the country, feedback and contributions from scientific communities and research groups are highly appreciated.



Lesser Flamingo Congregation at
Sewri-Mahul Mudflat, Mumbai

Hexavalent Chromium [Cr (VI)]: Yellow water Pollution and its Remediation

Alok Prasad Das^{1,*}, and Susmita Mishra²

¹Centre of Biotechnology, SOA University, Bhubaneswar, Orissa, India

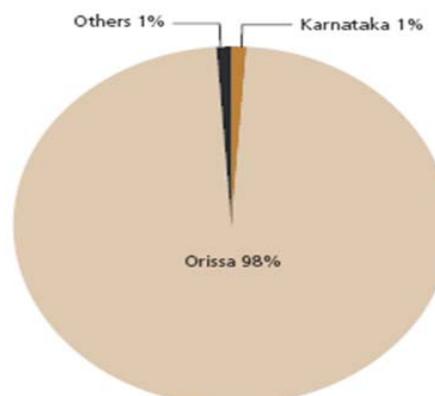
²National Institute of Technology, Rourkela, Orissa

*Email: alok1503@gmail.com

Introduction

Effluents from textile, leather, tannery, electroplating, galvanizing, dyes and pigment, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contains considerable amounts of toxic metal ions. These metal ions from metal mining pose problems to the water environment by discharging mine water from underground and open pit mines (Moncur et al 2005). Cr (VI) is toxic, carcinogenic and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology. They cause physical discomfort and sometimes life-threatening illness including irreversible damage to vital body system (Malik, 2004).

It is one of the world's most strategic, critical & highly soluble metal pollutant having wide range of uses in the metals and chemical industries (Kotas and Stasicka 2000, Das and Mishra 2008). Chromium exists in the environment in several diverse forms such as trivalent Cr (III) and hexavalent Cr (VI), of which hexavalent chromium Cr (VI) is a so-called carcinogen and a potential soil, surface water and ground water contaminant (Cervantes et al., 2001). A slight elevation in the level of Cr⁶⁺ elicits environmental and health problems because of its high toxicity (Sharma et al., 1995), mutagenicity (Nishioka, 1975) and carcinogenicity (Venitt and Levy, 1974). Whereas its reduced trivalent form, (Cr³⁺) is less toxic, insoluble and a vital nutrient for humans. Due to its toxicity stringent regulation are imposed on the discharge of Cr (VI) to surface water to below 0.05 mg/l by the U.S. EPA (Baral et al., 2002) and the European Union, while total Cr, including Cr (III), Cr (VI) and its other forms to below 2 mg/l.



Source: Anon, 2005, Indian Minerals Yearbook 2005, Indian Bureau of Mines, Nagapur, pp 52-13

Fig 1. Pie chart showing In India, almost 98 % of the chromite reserves is present in Orissa, mostly in the Sukinda valley of Jajpur. The country has total resources of around 179 MT, comprising of 47 MT of reserves.

Many such ferro alloy industries and chromite mines are located in the Sukinda area of Jajpur district in Orissa state. About 98% of India's chromite ore deposits are present in this region. (ENVIS Newsletter, 2007). Mining activity in this region generate around 7.6 million tonnes of solid waste in the form of rejected minerals, overburden material/waste rock and sub grade ore. Due to the seepage of water from the dumped waste the nearby water stream shows contamination due to Cr (VI) much above their permissible limits. OVHA (Orissa Voluntary Health Association) reports contaminations in the different water bodies of Sukinda area such as wells, ponds rivers etc. Here the water had become so contaminated by hexavalent chromium that the water turned yellow in colour. The OVHA reported health hazards due to Cr (VI) contamination leading to death. The main diseases includes, "gastrointestinal bleeding, tuberculosis asthma Infertility, birth defects, and still births".

The survey report further mentioned that villages at a distance of less than one km from the sites were the worst affected, with 24.47 per cent of the inhabitants found suffering from pollution-induced diseases (ENVIS Newsletter, 2007). Currently the effluents are treated with ferrous sulphate method that suffers from precipitation, so additional treatment methods to remove those are sorted. Hence our investigation would involve a means to reduce Cr (VI) from the effluent in a cost effective and eco-friendly manner. Thus our present study uses potential indigenous microbial strains for treatment of industrial and mining effluent that may be suitable for biological treatment of Cr-contaminated waste of Sukinda mines. This study proposes a remediation route for detoxification of Cr (VI) using an indigenous microorganism.

Methodology

Soil & Water samples were collected from different locations of the chromium deposited and contaminated site located at Sukinda mines and the physico-chemical characteristics of the samples was carried out.

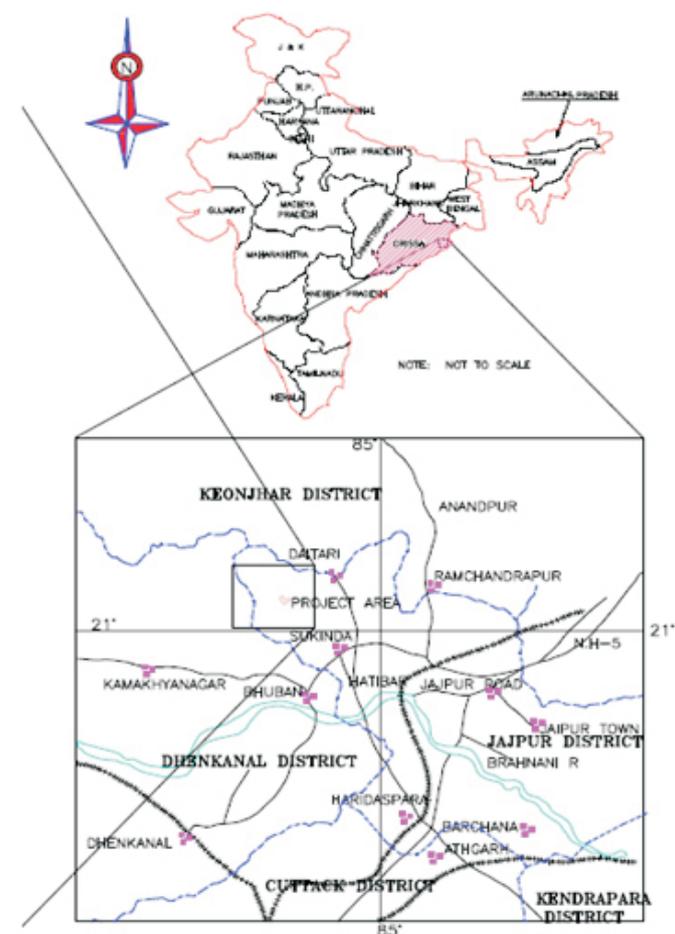


Fig 2. Map of Sukinda area

Scanning Electron Microscopy-Energy Dispersive X-ray analysis (SEM-EDX) evaluated to understand the morphology, elemental composition and particle density of the chromium contaminated soil sample. Bacterial strains, resistant to Cr (VI), were isolated from the soil using the serial dilution technique in nutrient agar medium. Three isolates are tested for their chromate tolerance at different concentrations (25, 50, 75, 100l/ml) of hexavalent chromium supplemented as $K_2Cr_2O_7$. A single strain was capable of growing at this condition & was selected for further experiments. Based on nucleotides homology and phylogenetic analysis (16S RNA sequencing) the Microbe was detected to be *Brevibacterium casei* (GenBank Accession Number: EU781952). Optimization of the process parameters such as p^H , temperature and media are carried out. The Cr (VI) reduction potential of the selected strain was directly checked in the Sukinda chromite mining effluent sample. Chromate-reducing activity was estimated by using the Cr (VI)-specific colorimetric reagent 1,5-diphenylcarbazide (DPC), at 540 nm.

Results and Discussion

The soil collected from the contaminated site located at Sukinda mines are used for the isolation of Cr (VI) bio-transforming bacteria containing 25.9 mg/g of soil and total Cr 9 ± 12 mg/g of soil. The result is consistent with the chromium values as found in the soil. The serial dilution technique was adopted for the isolation of the bacterial strain. The bacterial colonies that appeared on the PYE agar plates were isolated and further purified on the same media by spot inoculation technique.

The best chromium tolerant strain was isolated and tested for its ability for bio-transforming of chromium from the chromium ($K_2Cr_2O_7$) medium.



Fig 3. Isolates are tested for their chromate tolerance at different concentrations Cr (VI) in solid agar medium.

The SEM-EDX was used in its most common mode the emissive mode. Magnification: 10000 X, Accelerating voltage (kV): 15.00, Process time: 5, Detector: Silicon, Window: SATW, Elevation (deg): 35.0. The other parameters are as follows, Limit of detection, 5,000 accuracy, 10%; energy, 15 kV (accelerating voltages, 1240 kV) and spatial resolution, 5 nm (theoretical).

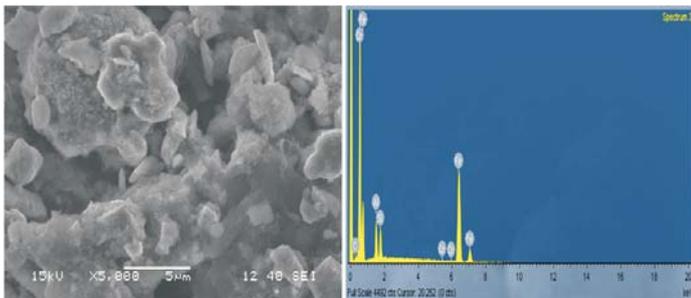


Fig 4 and 5. Scanning Electron Microscopy-Energy Dispersive X-ray analysis (SEM-EDX)

Based on nucleotides homology and phylogenetic analysis the Microbe was detected to be *Brevibacterium casei* (GenBank Accession Number: Eu781952).

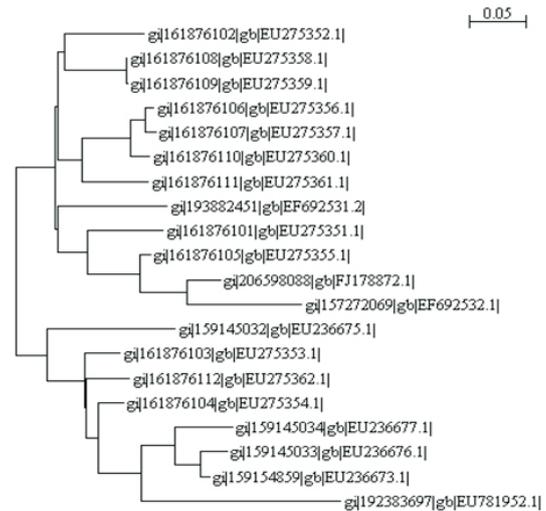


Fig 6. Phylogenetic tree of *Brevibacterium casei* with similar sequence from NCBI genbank

As evident from the experimental values, the suitable temperature and p^H for hexavalent chromium reduction was found to be $30^\circ C$ and 7 respectively. [Fig 7 & 8].

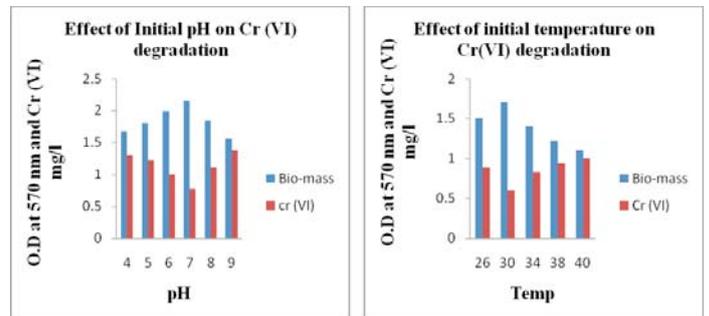


Fig 7 & 8. Effect of Initial pH and Temperature on Cr (VI) degradation kinetics at initial Cr (VI) concentration-50mg/l, Agitation-200rpm, inoculum volume-1ml

The Cr (VI) reduction ability of the bacteria was growth dependent & the *Brevibacterium sp.* reduces nearly 50 mg/L of Cr (VI) during degradation experiment in 10-12 hours. It is evident from the experiment that the time required for Cr (VI) degradation varies with initial chromium concentration. We can also find three different stages of degradation with initial rapid stage followed by slow rate and finally degrades at a till slower rate. Initial rapid degradation is observed within 8 hrs for varying chromium concentration. At lower chromium concentration equilibrium is achieved within 12 hours with 99% removal [Fig-9].

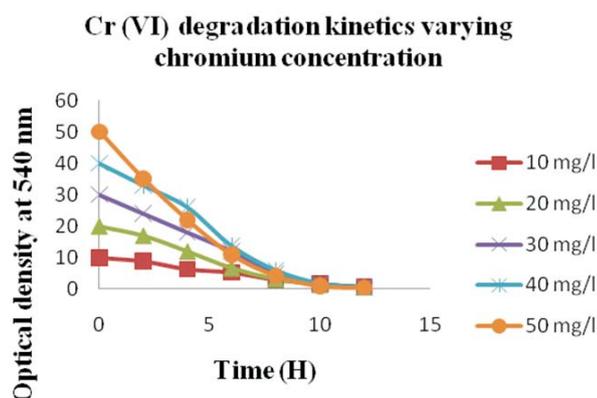


Fig-9: Cr (VI) degradation kinetics varying chromium concentration

Hence we can see that maximum reduction is observed within 12 hrs at initial chromium concentration 50 mg/L. This is attributed to high concentration gradient of chromium in the solution which is the main driving force for Cr (VI) degradation. The inoculum of the bacterial strains cultured overnight was used for this experiment. Culture flasks (150 ml) with a final volume of 100 ml supplemented with (10-50mg/L) of Cr (VI) were inoculated with 2ml of inoculums for 24 hour. In this experiment it is observed that lag phase is increasing with increased initial Cr (VI) concentration. It is basically due to inhibitory effect of higher chromium concentration on the growth of the organism. Each organism has a specific resistance at a specified growth condition. As the initial age of the inoculums was fixed at 24 hours the acclimatization period at varying chromium concentration will not remain same. Hence the following behavior is observed. The chromium-resistant bacteria isolate exhibited reduced bioaccumulation when cells were in stationary phase. At higher concentrations the growth of the bacteria is inhibited due to fixed amount of inoculums for all the different concentration of Cr (VI) considered in the experiment.

Conclusion

Our studies allow the following conclusions. The existence of chromium resistance bacterial strains isolated from anthropogenically chromium percolated ecosystems.

It is seen that the Identified species *Brevibacterium casei* can effectively degrade Cr (VI) upto 99% in 12 hrs at neutral pH & temp 30°C. This needs further research as the resistance potential of these organisms indicates the possibility of their exploitation in chromium & other heavy metal bioremediation in the future.

This needs further research as the resistance potential of these organisms indicates the possibility of their exploitation in chromium & other heavy metal bioremediation in the future.

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Communication, Education, Participation and Awareness (CEPA) Programme

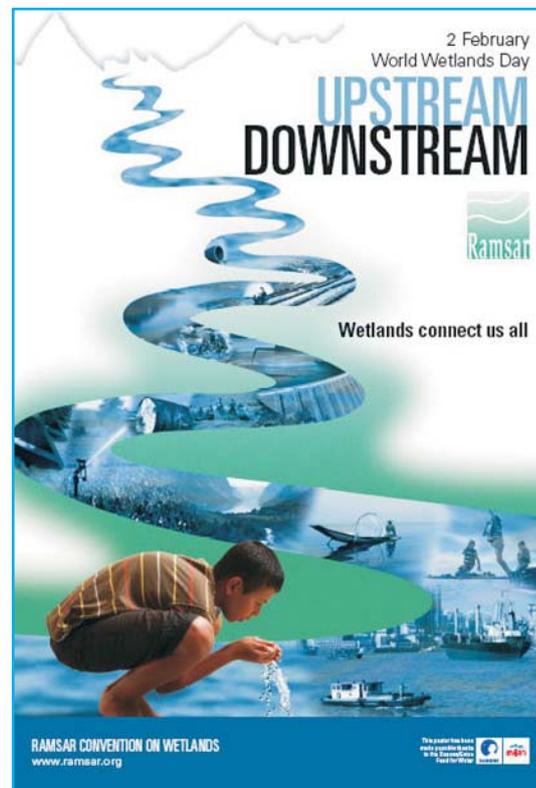
World Wetlands Day Celebration in Uppalapadu, Guntur District, Andhra Pradesh on 2nd February 2009

The Salim Ali Centre for Ornithology & Natural History (SACON), Deccan Regional Station, Hyderabad, as a part of its Environmental Information System (ENVIS) activities, celebrated World Wetlands Day on 2nd February 2009 at the famous Uppalapadu wetlands, Guntur district of Andhra Pradesh. More than 100 school children and several academicians, scientists, policy makers from a number of organizations took part in the event. This event was celebrated in collaboration with several organizations, Forest Department, J.P.High School and Guntur Railway Division.

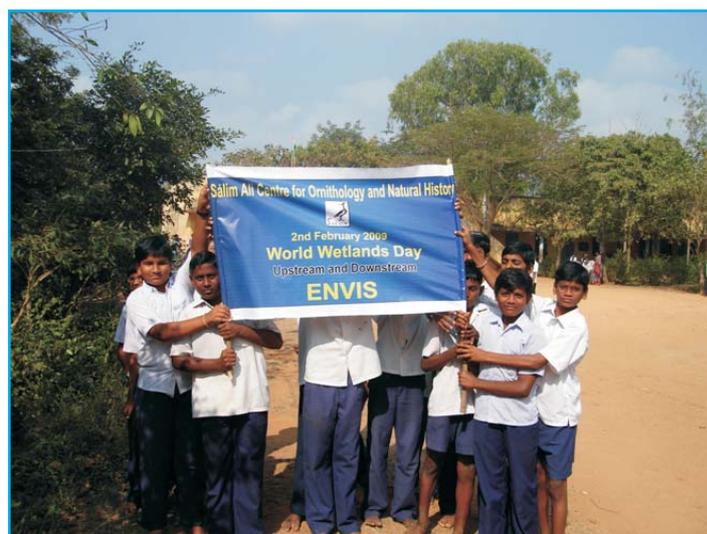
Wetlands are among the least protected ecosystems in developing countries, and India is particularly vulnerable to their degradation and loss. There are thousands of lakes, ponds, marshes, lagoons, estuaries, backwaters and mangrove swamps that are vital to the country's water needs, food production and biodiversity, but environmental policy has largely failed to acknowledge their contribution. The Uppalapadu, a fresh water tank situated in Uppalapadu village under Village Council (Gram Panchayat) revenue land of Guntur district, Andhra Pradesh. This place is very important for the globally threatened Spot-billed Pelican (*Pelecanus phillippensis*) species. Uppalapadu is only 8 km from Guntur Town.

The morning started with the bird watching programmes with school children at Uppalapadu wetland area. More than 100 school children from different schools were present at the event. All the students learnt about food habits and migration in birds but also water pollution, and the methodology for lake restoration. The children's programme was followed by an official function commemorating World Wetlands Day. Mr. Srinivas, DFO, Guntur, Dr. S. N. Prasad, Scientist & Head, SACON Deccan Regional Station, Hyderabad, Dr. Lalitha Vijayan, Scientist, SACON, Coimbatore, Railway Divisional Manager, Guntur, Principal, J. P. High School, Miss N. Sheeba, Research Scholar, SACON were also presented for this programme. All delivered their talk about the importance of wetlands and the role wetlands can play to

mitigate several problems. The programme culminated with a pledge to protect wetlands taken by all guests, officers and students present on the occasion.



WWD POSTER - 2009



Wetland Day Celebration at Uppalapadu,
Guntur, Andhra Pradesh

About SACON's initiatives to conserve the wetlands

SACON is one of the centres of excellence of the Ministry of Environment and Forests (MoEF), Government of India. SACON's mission is "To help conserve India's biodiversity and its sustainable use through research, education and peoples' participation with birds at the centre stage". The scientific staffs are organized into the division of Avian Ecology, Wetland Ecology, Terrestrial Ecology, Ecotoxicology, Environmental Impact Assessment, and Conservation Biology. The Environmental Information system (ENVIS) Centre on wetland ecosystems has been established at the SACON, Coimbatore supported by the MoEF, Government of India to facilitate generation and dissemination of information on various facets of wetland ecosystems.



Prize distribution ceremony
At J.P. High school



School children attending the
WWD function



Guests/Dignitaries attending the
WWD function

The Centre will work towards fulfillment of the following objectives: -

- Database creation on Wetland Ecosystems to be added on website with regional language interface.
- To establish and operate a distributed clearinghouse to answer and channel queries related to the allocated subject.
- To establish linkages with information users, carriers and providers from government, academic, business and Non-Governmental Organizations including that with ENVIS.
- Identification of information/data gaps in the specified subject areas and action taken to fill these gaps.
- Publication of ENVIS newsletters for dissemination of wetland Information.

For further information on wetlands of India, please visit our site (<http://www.wetlandsofindia.org/>)

“ Upstream Downstream : Wetlands connect us all ”



Spot-billed Pelicans nesting on the top of
the plants near Uppalapadu lake



Congregation of birds at
Uppalapadu Wetland



Query Form

Name: _____
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Your views on Scope of improvement: _____

Any additional comments: _____

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Chiranjibi Pattanaik, Senior Program Officer
Santosh Gaikwad, Information Officer
Madhu Routhu, Web Assistant

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Environmental Information System (ENVIS) Centre,
Salim Ali Centre for Ornithology & Natural History (SACON)
Anaikatty-641 108
Coimbatore, Tamil Nadu

Contact Us

SACON, Deccan Regional Station,
12-13-588/B, Nagarjuna Nagar Colony,
Tarnaka, Hyderabad-500 017 (Andhra Pradesh)
Tel/Fax: +91-40-27150328
(Email: sacon.wetland@gmail.com)

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To

