Half yearly report (2009-10) of NAIP sub-project on,"Arsenic in Food-Chain: Cause, Effect and Mitigation" under Component – 4

i) Project details

a) **Subproject title:** Arsenic in Food Chain: Cause, Effect and Mitigation

b) **Lead Institution:** Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, 741252.

c) Partners:

i) West Bengal University of Animal & Fishery Sciences, Kolkata

ii) Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar.

iii) Indian Veterinary Research Institute (ERS), Kolkata.

iv) Central Inland Fisheries Research Institute, Barrackpore.

v) D.N. Guha Majumdar Research Foundation, Kolkata.

d) Objectives:

i) To study genetics of arsenic tolerance, physiology and transport mechanism in rice

ii) To identify microbes which could be used for soil amelioration.

iii) To characterize the path of arsenic in food-chain along with mitigation options.

iv) To determine the adverse effect of arsenic on human health through food-chain.

e) **Total Budget of the sub-project** : Rs. 673,29,005.00

f) **Date of Sanction** : May 4th , 2007

g) **Subproject website address (URL)** : naip-arsenic.bckv.edu.in

ii) Technical progress (during April 1st to September 30th 2009 and cumulative)

a) During the first half of this year

Deliverables	Targets	Achievements	Remarks
Linkage analysis for the markers with the QTLs responsible for arsenic-accumulation in straw and grain. *	SSR markers linked with low arsenic accumulation in grain & straw	53 Polymorphic SSRs among the parents are identified.	Their linkage with low arsenic accumulation will suppose to be delivered after 3rd year as differential arsenic accumulation status of RILs (data for two years) will only be available after analysis of Kharif-2009 rice-harvest.
Understanding pathway of arsenic transport in rice, based on available information on Arabidopsis.*	Identification of genes for arsenic transport mechanism in rice	Expression analysis of Multidrug and toxic compound extrusion transporter (MATE), efflux transporter of silicic acid (LSi_2), Arsenate reductase gene (ACR2.1) and Histone H3 genes has been assessed (in stem and root of grain filling stage) of High (0.76mg/kg)	Seven fold higher expression (obtained from real time PCR data) than that of high accumulating genotypes. MATE might be one of the candidate genes for restricting arsenic loading (to the tune of

		and low arsenic (0.32mg/kg) accumulating rice genotypes (Two each) grown in arsenic contaminated (> 0.48mg/kg of soil) farmer's field.	50% than that of high accumulating genotypes) into the spikelet.
Identification and characterization of arsenic transforming microbes.*	Identification of microbes isolated from soil, pond water and pond sediments. Isolation target was 1000 species.	Phenotypical identification done for 41 bacterial species. Most of the strains isolated from aquatic, sediment and surface soil (0-15 cm) samples. Thirty-five strains were gram negative, among them, 8 were monococcus, 10 staphylo coccus, 12 rod shaped in chain form, 5 were short rod shaped species. Six strains were gram positive.	Among the identified species 19 strains are found to arsenite oxidizing and 10 strains are arsenate reducing.
Fish physiology, clinical pathology and biochemistry of fish from affected zones, as well as, through controlled laboratory experiment.*	Assessment of health status of one fish species (*Labeo rohita)* exposed to arsenic (5-60 ppm) under laboratory condition and their proteomic analysis	In a lab based experiment fingerlings of *Labeo rohita* exposed to 10 levels (5-60 ppm) of arsenic solution for 10 days. After ten days pigmentation on skin, aberration of fins, development of scar, *etc*. were noticed in fingerlings exposed to solution with >5 ppm arsenic. Processed tissues for histopathological studies. Proteins from muscle, liver, kidney, and gill were analyzed by SDS-PAGE for identification of marker proteins.	Total mortality was noted within 12.75 & 113.25 hr. when exposed to water with 60 & 15 ppm total arsenic. This study gave an idea about the lethal dose of arsenic to fingerlings of *Labeo rohita a m*ajor Indian carps. Such data is not available. SDS-PAGE results are under analysis
Generation of fish (muscle) tissue 2-D proteome maps *Labeo rohita* species collected from ponds in arsenic affected area and compared with carps collected from ponds in control area.	Selection of unique protein spots of muscle proteome through mass spectrometry. Number of spots will be decided based on image analysis results for	Generated Muscle proteome maps of *L. rohita* , and analyzed by 1- and 2-D gel electrophoresis. A visual comparison of the proteome maps shows some additional protein spots (p53) in the samples from arsenic-contaminated zones. These orphan spots will have to be identified by PMF and mass spectrometry to understand its role and function	Muscle is the major biomass from fish that is consumed and any pathophysiological changes associated with arsenicosis should be reflected in the muscle protein. This findings proof that, like human chronic exposure of arsenic is carcinogenic to fish also. n profiles.

Study of clinical symptoms, arsenic level in blood, milk, meat, urine, etc., biochemical, haemogram, and immune status on livestock.*	Analysis of arsenic status of feed, drinking water, blood, milk, hair, urine and faeces of 30 cows and egg of 10 ducks belonging to the families having arsenicosis patient(s) from each arsenic affected villages were selected. Record of clinical status like Debilated, rough body coat, chronic diarrhea of selected cows.	Arsenic status of straw, drinking water, hair, urine, feces, milk, egg albumin and egg yolk were respectively in the range of 0.202–6.315, 0.001–0.391, 0.212-10.948, 0.012-0.428, 0.049-1.277, 0.001-0.631, 0.002-0.246 and 0.017-0.328. No clinical symptoms of arsenic pollution were detected from the animals.	Relationship between arsenic content of straw with that of hair and feces were respectively 0.4 and 0.3.
Study of clinical symptoms, arsenic level in blood and tissues, etc., biochemical haemogram, immune status and candidate gene mapping of rodent.	Study of oxidative stress in relation to arsenic deposition in different organs (liver, kidney, spleen) and blood of rats.	Rats drink water for 12 weeks contains 20 ppm sodium arsenite. In case of lipid peroxidation there was significant increasing trend from 4th wk onward for all tissues like liver, kidney, spleen and blood. In spleen and blood SOD activity reduced from 8th wk onward but catalase activity of spleen was increased on 8 wk onward. Catalase activity of spleen was increased up to 8 wks and it was significantly (p<0.01) reduced on 12 wks of arsenic exposure. In blood, catalase activity was decreased at 4^{th} wk and maintained the trend upto 12 wks.	
Therapeutic measure to mitigate the effect	Ameliorative approach using Mushroom lectin	Monolayer of rat hepatocytes culture was treated with 5 micromol arsenic and	

in monolayer of rat hepatocytes culture under in vitro system.	(ML) and ascorbic acid (AA) to normalize the immuno disruptive effects and oxidative stress in rat hepatocytes *in vitro*.	trteated with 50 micro gram per ml of ML and 10 micrmol ascorbic acid. The cell culture was maintained for 5 days with arsenic exposure and treatment. Arsenic decreases cell proliferation index, SOD & CAT activity, increases NO production and LPO level. Status of cell proliferation, SOD & CAT activity increased but NO production was not significantly increased, when hepatocytes of rats were treated with ML.	
Study of oxidative stress of human population.	Analysis of human blood samples (received from DNGMRF) for oxidative stress parameters	Till date 66 human blood samples received from DNGMRF and analyzed for superoxide dismutase (SOD) activity of RBC and Lipid peroxidation (LPO) in plasma.	
Optimum irrigation regime and arsenic content in rice and maize grain.*	Quantification of relationship between arsenic status in grain with arsenic status of soil, total arsenic added through irrigation water and total ET.	In case of boro rice 'r' value of grain arsenic status against amount of arsenic added through water and soil arsenic status at harvest were 0.79 and 0.63 respectively. However, against maize grain 'r' values were respectively 0.76 and 0.85. 'r' value between AET and grain arsenic status were 0.78 and 0.83 respectively for boro rice and maize crop.	Single variety was taken as test crop for both boro rice (Gontra Selection -3) and maize (Dekalp all-rounder). Arsenic status of irrigation water was 0.163. At harvest soil arsenic status in rice and maize fields were respectively 21.73 – 46.25 and 12.2 – 20.9 mg /kg soil.
Retention – release characteristics of arsenic from soils in presence of different anions and at varying Eh/pH conditions.	To study the specific arsenic sorption-desorption parameters with soils and reflection thereof ,if any ,with anions (phosphate, nitrate, sulphate, borate,	The arsenic sorption – desorption parameters with soils are being fitted with some empirical models(Freundlich, Langmuir and Tempkin) . The method standardization for Eh/pH with arsenic in aqueous phase is in progress.	

	molybdate) and changing redox potential of the solution.		
Analysis and interpretation of health parameters in humans under arsenic exposure through diet.	Progressing of disease manifestation in arsenicosis patients	Completed clinical analysis including ECG, X'ray, USG, Blood test of 308 participants covering all three cohorts. Conducted 36 lung function test and drug trial on 100 cases of cohort 1. Arsenic exposure through water gives moderate "r" value with arsenic status of hair and urine of 308 cohort populations.	A drug trial with Indigenous drug Curcumin has been started on Arsenicosis patients

* Study carryout from first year.

b) **Cumulative so far from the beginning of the sub-project**

Deliverables	Targets	Achievements	Remarks
Differentiation of RIL population for arsenic uptake and identification of polymorphic markers.	i. Arsenic status in grain and straw of 100 RIL lines. ii. As many as polymorphic SSRs trough out the genome	i. Arsenic status in grain and straw of 80 RIL lines. ii. 53 polymorphic SSRs out of 170 SSRs tested.	
Isolation, identification and characterization of microbes capable of transforming arsenic to less toxic form.	Isolation and identification of soil and aquatic microbes from 1000 samples.	1030 bacterial strains were isolated. Oxidation/reduction of the isolates investigated by $KMnO_4$ and spectrophotometric methods. MIC value of isolates ranging from 175 – 275 mM for sodium arsenate and 10 – 17.5 mM for sodium meta arsenite	.
Status of arsenic contaminations in ground water, pond water and sediment of hatcheries.	Source and arsenic status of crop seed, organics, spawn, etc.	Significant amount of arsenic detected in water (35 – 88 ppb) and sediment (2.4 – 68.7 ppm) samples collected from hatcheries since contaminated ground water is the main source of water. Fish fingerlings also found moderately contaminated (up to 60 ppb).	
Effects of organics towards mitigation of arsenic into food chain from contaminated	To find out the role to play by the organics in terms of binding arsenic in soil	From the experiment of Humic/Fulvic (extracted from FYM and vermicompost) – arsenic complexation study the binding capacity range is 1.5-	The higher molecular weight of HA/FA extracted from FYM and

sources.	matrix.	2.3 which means 1.5-2.3 moles of HA/FA will combine with one mole of arsenic. The FYM and Vermicompost were collected from the arsenic affected area as developed by the local farmers with cowdung, litters, earthworm, vegetable wastes etc.	Vermicompost and the cage type conformation around arsenic ion because of the hydrophobic hydration, impart to the organo-arsenic complex the more stability.
Identification and finalization of cohorts. Standardisation of protocols.. Clinical examination of sample subject. Collection of biomarker samples	Selection of cohorts and its status	Identified 108, 100 and 100 population respectively in Cohort I, II and III. Completed Age-Sex matching. Analyzed 223,325 and 178 water, urine and hair samples.	

iii) Major innovations/achievements so far from the beginning of the sub-project

- Multidrug and toxic compound extrusion transporter (MATE) might be one of the candidate genes for restricting arsenic loading (to the tune of 50% than that of high accumulating genotypes) into the spikelet.
- *Vibrio diazotrophicus* was identified as arsenic oxidising bacteria by Biolog method with 91% probability. The bacteria was aerobic, Gram-ve, bacillus. In literature *Vibrio diazotrophicus* has been identified as a nitrogen fixing bacteria in marine environment. This is the first report of arsenic oxidation by *Vibrio diazotrophicus* collected from pond sediments.
- Arsenic exposure decreases cell proliferation index, superoxide dismutase (SOD) and Catalase enzyme (CAT) activity, increases Nitric Oxide production and Lipid Peroxidation level.
- Soil arsenic status influences arsenic content of maize grain to a greater extent (r = 0.85) than that of the total arsenic added to the crop field through contaminated irrigation water (0.76). In case of boro rice the 'r' values of grain arsenic against soil arsenic at harvest and total arsenic added through irrigation were 0.63 and 0.79 respectively.
- Organic amendments efficiently reduce arsenic availability in soils. Organics made complex of As with humic acid (HA) / Fulvic acid (FA) fractions (☐ to the tune of 0.43 – 0.66 mole As : 1 mole of HA/FA).
- Food constituted Raw Rice (Boro), Pulses, Fish, Egg, Milk & Vegetables, collected from study area. Data of mean As value of some of these samples have been extrapolated in the As value of food intake by the participants. Out of these, rice constituted the major contributor of As exposure through food. Extrapolating all these data, we observed As exposure through food ranged up to to 925 µg/day and urinary excretion ranged up to 540 µg/l in 122 study subjects who were drinking As in water <50 µg/l. There was some correlation between As intake through food and As in urine (p,<0.01).

iv) Information on results framework (as per the Component)

Component-4: Performance indicators for the period April 1st to September 30th 2009

Indicator	Baseline Value	Performance
Number of overseas visits/trainings	Under process	
Number of papers in high impact scientific journals (factor 5 and above as per NAAS system)	List given below*	
Number of patent applications filed	Nil	
Number of major methodological tools developed which are really novel	Nil	

***Paper published**

1. Pan D, Bera A K, Das S, Bandyopadhyay S, Rana T, Bandyopadhyay S, Das SK, Bhattacharya, D. 2009. Use of zinc chloride as alternative stimulant for in vitro study of nitric oxide production pathway in avian splenocyte culture. **Mol Biol Rep**. [In press]. NAAS Impact Factor 7.8, International impact factor 1.75

2. Guha Mazumder DN. Chronic Arsenic Toxicity and Human Health, Indian J Med Res 128, pp 436-447, 2008.

3. Majumder KK, Guha Mazumder DN, Ghosh N, Ghosh A and Lahiri S. Systemic manifestations in chronic arsenic toxicity in absence of skin lesions in West Bengal, Indian J Med Res 129, 75-82, 2009.

4. Tanmoy Rana, Samar Sarkar, Tapan Kumar Mandal, Subhasis Batabyal, Bakul Kumar Datta, Sumanta De, Sourav Sikdar. Study on the effect of toxicity under highly arsenic prone zone in Nadia district of West Bengal in India. Nature Precedings: hdl:10101/npre.2009.2992.1 : Posted 28 Mar 2009.
5. T. Rana, S. Sarkar, T. Mandal, K. Bhattyacharyya & A. Roy : Arsenic residue in blood, urine and faeces samples from cattle in the Nadia district of West Bengal in India . The Internet Journal of Veterinary Medicine. 2008 Volume 4 Number 1
6. T. Rana, S. Sarkar, T. Mandal & S. Batabyal : Haematobiochemical profiles of affected cattle at arsenic prone zone in Haringhata block of Nadia District of West Bengal in India . The Internet Journal of Hematology. 2008 Volume 4 Number 2

Submitted for publication

1. Guha Mazumder DN, Ghosh A, Majumdar KK, Ghosh N, Saha C, Guha Mazumder RN. Arsenic contamination of Ground Water and its Health Impact on Population of District of Nadia, West Bengal, India. Ind. J of Community Medicine.

2. Ghosh A, Guha Mazumder DN, Mistry G, Majumdar KK, Ghosh N, Saha C, Nandy A. Cardiovascular effect of chronic of Arsenic Exposure A Case control study in West Bengal, India. Envl. Health. Presp.(USA).

v) Financial Management

a) During the year

Budget heads	Total Outlay (Rs. lakhs) for the year	Released so far including the unused amount from the previous year (Rs. In lakhs)	Amount revalidated (Rs. lakhs)	Expenditure (Rs. in lakhs)	Percent Utilization of the column (3+4)	Remarks
1	**2**	**3**	**4**	**5**	**6**	**7**
TA	5.00000	2.63945	0.09541	0.97908	35.80	
Workshop	2.50000	3.38470	0.00000	0.00000	0.00	CIFRI and DNGMRF will organize workshop during the 2nd half of current financial year
Contractual Services	39.83800	19.32467	0.00000	18.56908	96.09	
Operational Expenses	23.50000	11.56002	0.23418	10.36356	87.87	
Training	1.00000	0.50000	0.00000	0.00000	0.00	
Equipment	0.00000	10.50344	0.80535	1.73634	215.6	Rs.0.6 and 0.33099 lakh transferred to DNGMRF from BCKV and UBKV
Furniture	0.00000	0.00407	0.00653	0.00000	0.00	
Works/ Renovation	0.00000	0.00000	1.00000	0.00000	0.00	
Others	2.00000	4.10922	0.00654	0.00000	0.00	
Institutional charges	4.67160	2.32083	0.00000	2.32083	100.00	
Total	78.50960	54.34640	4.14801	33.96889	58.07	

b) Cumulative

Budget heads	Total Outlay (Rs. lakhs) for the whole sub-project	Total amount released (Rs. lakhs)	Total amount used (Rs. lakhs) till date from the start of the sub-project	Per cent of the total outlay used till date from the start of the sub-project	Remarks
TA	23.91880		4.86588	20.34	
Workshop	10.00000		1.22541	12.25	
Contractual Services	179.1387		62.38392	34.66	
Operational Expenses	126.10890		47.3909	37.58	
Training	7.00000		0.00	0.00	Under process
Equipment	281.68260		269.36906	95.92	
Furniture	6.65000		6.6394	99.84	
Works/ Renovation	4.00000		2.95609	73.90	
Others	11.00000		3.35424	30.49	
Institutional charges	23.79105		9.97188	41.91	
Total	673.29005		408.15678	60.62	

vi) Procurement

a) During the year

Major items (Above Rs. 5.0 Lakhs)	Budget	Utilization (%)	Remarks
Nil			

b) Cumulative

Major items (Above Rs. 5.0 Lakhs)	Budget (Rs. In lakh)	Utilization (%)	Remarks
LC-ICP-MS	130.00	97.24	BCKV
Table top centrifuge	14.00	91.44	BCKV
BGI, HPLC system	12.00	74.00	BCKV
Generator	9.00	98.66	BCKV
Phase Contrast Microscope	12.00	96.16	BCKV
UV-VIS Spectrophotometer	9.00	101.62	BCKV
AAS	25.00	103.41	WBUAFS

vii) Baseline survey report submitted **No**

viii) If not submitted yet, please state the stage at which the baseline survey report is , likely date of submission and reasons for delay: Data compilation and writing stage, submitted before 31.12.2009. Non functioning of ASS cause the delay in analysis of arsenic in collected samples.

ix) Project website/webpage being updated regularly **Yes**

x) Please give the reasons if you are not updating the website regularly or if you do not have a website till date

xi) Media products developed/disseminated Nil

Products (CDs, Manuals, Bulletins, Paper clippings etc.)	Copies Supplied

xii) Implementation problems/issues and suggestions

Problems	Suggestions for remedy

xiii) Have you circulated the report among the CAC members and taken the approval of the Chairman CAC? Draft copy of the report sent to the Chairman and two external members of the CAC, Consortium Leader and the Director of Research, BCKV. Ten days time was given to them.