Submission of Concluded Research Project
(To be submitted separately for each project)

1. Project code and title:

PPS-3598: Arsenic contamination in mulberry sericulture of Bengal plain and its alleviation through application of zinc in soil

2. Names of the Project Investigators (including coordinator in case of collaborative projects)

Investigator	Name	Designation	Time Spent (%)
Executive Authority	Dr. Kanika Trivedy	Director	-
Coordinator	Dr. Monica Chaudhuri	Scientist-D	Up to 31.12.2017
Principal Investigator	Dr. Vijay, V.,	Scientist-B	70 %
Co-Investigator	Dr. Ranjit Kar	Scientist-D	20 %
Co-Investigator	Dr. R. Mahesh	Scientist-B	5 %
Co-Investigator	Mr. Gopal Chandra Das	Scientist-D	5 %

3. Duration (Date of Start) - (Scheduled Date of Completion):

3 year (01.11.2016 – 31.10.2019)

4. Name(s) of the Institute(s) and Address:

Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore – 742101, Murshidabad, West Bengal

5. A list of Objectives / Goals (clearly indicating how far they have been achieved; indicating the difficulties / reasons in case of achievement gap):

S.No.	Objectives	Achievement
1	To investigate the extent of arsenic load/accumulation in irrigation water-soil-mulberry plant-silkworm larva continuum under mulberry vegetation.	• 100 % • Found bioaccumulation of arsenic in water-soil-mulberry-silkworm continuum at farmers' field.
2	To formulate dose of zinc for alleviating arsenic pollution in	In spite of bioaccumulation of arsenic in water-soil-mulberry-silkworm continuum, no

	mulberry sericulture by application of zinc in soil wherein it is deficient.	hazardous effect was found on silkworms reared at farmers' filed using mulberry leaves grown on arsenic contaminated soil containing available arsenic up to 21.13 mg kg^{-1}. Therefore 47th RAC advised to conclude the project.
3	To validate the efficacy of laboratory findings by conducting the appropriate field trials.	

6. Introduction:

Groundwater from many tube wells in the Eastern part of the river Bhagirathi/ Hoogly are highly contaminated with arsenic (As) (i.e. > 0.05 ppm) (http://www.soesju.org/arsenic/wb.htm; http://maps.wbphed.gov.in/arsenic/index.html). Agricultural soils of sericulturally important districts (Murshidabad, Malda and Nadia) falling under this area are also in the trap of arsenic contamination from the arsenic loaded groundwater as a source of irrigation affecting soil quality. As per SOES report, out of 26, 14 and 17 blocks of Murshidabad, Malda and Nadia districts, groundwater arsenic contamination in Jalangi, Kaliachak-II and Karimpur-I is reported to be maximum (50.9, 57.5 and 27.5 % of samples studied having the said contamination) with >0.05 mg l^{-1} arsenic and the maximum concentration of arsenic found in these blocks are 2.0, 1.9 and 1.4 mg l^{-1}, respectively.

For ascertaining the ill effect of arsenic on sericulture, if any, the present study was proposed. Wherein, such effect would be ascertained by monitoring arsenic load *in situ* in irrigation water-soil-mulberry leaf-silkworm larva samples from farmers' sites in arsenic contaminated area.

7. Methodology Adopted:

i. *Selection of study area*: The study area/ village was selected after thoroughly referring GIS based maps for arsenic affected areas of West Bengal available at Public Health Engineering Department (PHED), Government of West Bengal website (http://www.soesju.org/arsenic/wb.htm; http://maps.wbphed.gov.in/arsenic/index.html).

ii. *Selection of farmers field*: From the selected village, arsenic load in irrigation water of randomly selected 32 tube wells were surveyed. Based on the findings, three farmers crop (under irrigation) from each of the five wells [ranked as High, Medium, Moderate, Low and Least level of arsenic (As) contamination] were chosen to study the *in situ*

affect of As contamination, as well as the extent of bio-magnification of As, if any, in the groundwater-soil-plant-silkworm system.

iii. *Analysis of arsenic in samples*: Arsenic load in irrigation water was analysed using rapid arsenic test kit (MQuant^{TM}) and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) at CSR&TI, Berhampore and NCCCM, BARC, Hyderabad, respectively. Arsenic in soil, mulberry leaf (pooled oven dried powder of 2nd, 5th and 8th leaf of 5 plants from each farmer's crop) and silkworm larva (pooled oven dried powder of 10 Vth instar 3rd – 5th day larva from each farmer's crop) samples were analysed using AAS-HG [Perkin Elmer AANALYST 200 atomic absorption spectrophotometer (Perkin Elmer, USA) coupled with a same make hydride generator (FIAS 400)] at Arsenic Research Group, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal (Das *et al.*, 2016; Sarkar *et al.*, 2012; Huang and Fuji 1996).

8: Observations / Results duly indicating the output in terms of adding to knowledge; know-how / new packages/ practices / processes /products / innovations developed and their utility and advantages; etc.,

i. *Selection of study area*: On the basis of a preliminary geographic information system (GIS) based survey reported by Public Health Engineering Department, Government of West Bengal on arsenic load in groundwater of various villages in West Bengal, Dakshin Debipur (24°54'15.4"N 88°01'30.1"E) in Malda district was selected as an widely arsenic affected area with 82% samples (n=40) containing >0.05 ppm As (fig. 1) (http://maps.wbphed.gov.in/arsenic/index.html).

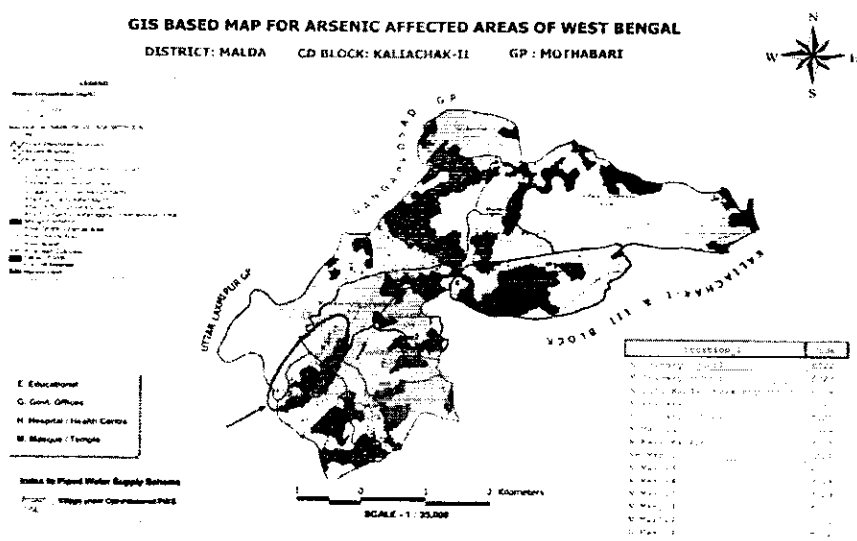

Fig. 1. GIS based map for arsenic affected areas of Mothabari gram panchayat of Kaliachak-II block in Malda district. Area circled and indicated by red arrow is Dakshindebipur village. (Source: http://maps.wbphed.gov.in/arsenic/index.html)

ii. *Arsenic concentration in studied samples*: All abiotic and biotic samples collected, processed and analysed in this study were found to be contaminated with varying concentration of arsenic (Table 1). Total arsenic (As) in 32 shallow tubewell water samples ranged from 0.0075 ± 0.0008 mg l^{-1} to 0.6692 ± 0.0135 mg l^{-1}. Available As in the 15 farmers' field soils ranged from 2.28 to 21.13 mg kg^{-1}. Bioaccumulation of As in mulberry leaf and silkworm ranged from 5.21 – 34.01 mg $kg^{-1}$and 0.004-0.035 mg g^{-1}, respectively. In general, the order of arsenic-abundance in irrigation water-soil-plant-silkworm continuum was observed as follows:

Water < Soil (available) <Silkworm (V^{th} instar larva) < Mulberry leaf

Table 1. Arsenic concentration in samples collected from seri-farmers of Dakshindebipur.

Sample ID	Water	Soil	Leaf	Silkworm
	Total As [mg l^{-1}]	Avl. As [mg kg^{-1}]	Total As [mg kg^{-1}]	Total As [mg g^{-1}]
F-10	0.60	3.55	28.67	0.008
F-15	0.60	11.64	15.10	0.007
F-17	0.60	9.56	20.00	0.026
Mean ± STDEV	**0.60**	**8.25 ± 4.20**	**21.26 ± 6.87**	**0.014 ± 0.011**
F-6	0.32	11.29	14.55	0.009
F-22	0.32	11.39	26.37	0.035
F-23	0.32	12.19	14.60	0.007
Mean ± STDEV	**0.32**	**11.62 ± 0.49**	**18.51 ± 6.81**	**0.017 ± 0.016**
F-4	0.15	21.13	5.21	0.012
F-5	0.15	15.09	18.03	0.009
F-28	0.15	4.67	21.05	0.029
Mean ± STDEV	**0.15**	**13.63 ± 8.32**	**14.76 ± 8.41**	**0.017 ± 0.011**
F-7	0.08	19.82	17.25	0.010
F-11	0.08	18.18	16.20	0.025
F-20	0.08	2.28	19.76	0.006
Mean ± STDEV	**0.08**	**13.43 ± 9.69**	**17.74 ± 1.83**	**0.014 ± 0.010**
F-3	0.007	3.04	23.93	0.004
F-12	0.007	18.81	34.01	0.007
F-25	0.007	11.06	14.01	0.026
Mean ± STDEV	**0.007**	**10.97 ± 7.89**	**23.98 ± 10.00**	**0.012 ± 0.012**

9. Discussion:

The survey shows that the total arsenic content of the thirty two shallow tube well water was in the range of 0.007–0.600 mg l^{-1} with a mean value of 0.160 mg l^{-1}, which is 16 times higher than the safe limit (0.01 mg l^{-1}) of arsenic toxicity as defined by World Health Organization (WHO) and Bureau of Indian Standards (BIS). Experimental results of the fifteen soils of the participant-fields showed the mean Olsen extractable arsenic (i.e plant available arsenic) concentration ranged between 2.28 and 21.13 mg kg^{-1}, with a mean value of 11.58 mg kg^{-1}, considerably higher than the critical limit (0.43 – 0.54 mg kg^{-1}) for rice cultivation but less than soil arsenic concentration (40 mg kg^{-1}) required to develop toxicity symptoms in leaf and root of rice plant (Golui *et al.*, 2017; Das *et al.*, 2013). Consonantly, no arsenic toxicity symptoms were found on the mulberry leaves. Tolerance of mulberry plants to high concentration of arsenic can be attributed to their perennial nature as well.

The total As content of mulberry leaves of the fifteen fields was in the range of 5.21–34.01 mg kg^{-1} with a mean value of 19.25 mg kg^{-1}. With a presumed life time (4 weeks) ingestion of minimum 16 gm leaf/larva, equivalent intake of As by a larva would be 0.08-0.54 mg As/larva, which is much higher than the minimum lethal dose of arsenic toxicity in silkworm (i.e. 0.04 mg As(V)/2 g larva weight) and provisional tolerable dietary intake of As for humans (0.00012 mg inorganic As/2 g body weight/4 weeks) as reported by Campbell (1926) and jointly assigned by FAO & WHO, respectively. However, the total As bio-accumulated in silkworm larvae were found to be in the range of 0.004–0.035 mg g^{-1} with a mean value of 0.015 mg g^{-1}. This approximate ten times difference between ingested (0.08-0.54 mg As per 2 g larva) and accumulated arsenic (0.008–0.07 mg As per 2 g larva) would be due to different bioavailability of inorganic and organic compounds of arsenic in leaves as well as excretion of waste products of metabolism as urine together with faeces (Mandal, 2017; Lakshmikantham *et al.*, 2013). The above calculations were derived from conjecture that: one disease free laying (dfl) contains 500 eggs or larvae and needs minimum 8 kg leaves for complete rearing (i.e. 4 weeks); fifth instar silkworms average weight equals to 2 g; and the entire As in leaf is present in inorganic form (Juhasz *et al.*, 2006; Campbell, 1926).

In spite of high level of arsenic contamination in water (0.6 mg As l^{-1}), soil (21.13 mg Avl. As kg^{-1}), mulberry leaves (34.01 mg As kg^{-1}) and silkworms (0.035 mg g^{-1}), the worms were appeared healthy without any symptoms of arsenic toxicity in terms of mortality or any unusual physical/ physiological challenge.

10. Inférence / Recommandations:

From the above study it can be ascertained that arsenic contamination has no hazardous effect on sericulture performed using mulberry plants grown on arsenic contaminated soils (i.e. up to 21.13 mg Avl. As kg^{-1}). Therefore, under present *in situ* circumstances, application of zinc for alleviation of arsenic in mulberry growing fields is not essential. Considering the above results, 47th RAC advised to conclude the project and present the same with proper justification in the forthcoming RAC meeting.

11. Applications made for patenting / commercialization if any:

Nil

12. References

Campbell, F.L., (1926) Speed of toxic action of arsenic in the silkworm. *The Journal of General Physiology.* ix, 433-443.

Das, I., Ghosh, K., Das. D.K., Sanyal, S.K. (2013) Assessment of arsenic toxicity in rice plants in areas of West Bengal. *Chemical Speciation and Bioavailability.* 25(3):201-208.

Das, I., Sanyal, S.K., Ghosh, K. and Das. D.K. (2016) Arsenic mitigation in soil-plant system through zinc application in West Bengal soils. *Bioremediation Journal.* 20(1), 24–37. http://dx.doi.org/10.1080/10889868.2015.1124062

Golui, D., Guha Mazumder, D.N., Sanyal, S.K., Datta, S.P., Ray, P., Patra, P.K., Sarkar, S., Bhattacharya, K. (2017) Safe limit of arsenic in soil in relation to dietary exposure of arsenicosis patients from Malda district, West Bengal- A case study. *Ecotoxicol. Environ. Saf.* 144:227-235. doi: 10.1016/j.ecoenv.2017.06.027.

Huang, P.M., and Fujii. R. (1996) Selenium and arsenic. In Methods of soil analysis. Part 3. Chemical methods, ed. D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston and M. E. Sumner, 811–818. Madison, WI: Soil Science Society of America, Inc., American Society of Agronomy, Inc.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., Naidu, R. (2006) In vivo assessment of arsenic bioavailability in rice and its significance for human health risk assessment. *Environ Health Perspect.* 114(12):1826-31.

Lakshmikantham, V. and Bharathi, D. (2013) Influence of Indole-3-acetic acid (IAA) on the nitrogenous end products of silkworm, *Bombyx mori* L. *Indian Journal of Sericulture.* 52:160-162.

Mandal, P. (2017) An insight of environmental contamination of arsenic on animal health. *Emerging Contaminants.* 3:17-22. doi.org/10.1016/j.emcon.2017.01.004

Sarkar, S., Basu, B., Kundu, C.K., Patra, P.K., (2012) Deficit irrigation: An option to mitigate arsenic load of rice grain in West Bengal, India. *Agriculture, Ecosystems and Environment.* 146, 147-152. doi:10.1016/j.agee.2011.10.008

13. Summary:

In this study, hazardous effect of arsenic on sericulture was investigated *in situ* by surveying arsenic load in irrigation water-soil-mulberry leaf-silkworm larva samples from farmers' crop grown on arsenic contaminated fields at Dakshindebipur village in Kaliachak block, Malda district. From the study, it was found that arsenic contamination has no hazardous effect on sericulture, performed using mulberry plants grown on arsenic contaminated soils (i.e. up to 21.13 mg Avl. As kg^{-1}). Therefore, considering additional economical and environmental burden, under present *in situ* circumstances, application of zinc for alleviation of arsenic in mulberry growing fields does not appear to be pragmatic.

15. Budget utilized (In lakh rupees): Rs. 1,49,613=00 (Rupees one lakh forty nine thousand six hundred and thirteen only)

A. **Non-Recurring** (e.g. equipments, accessories etc.):

Sl. No.	Item	2016 - 2017		2017 - 2018		2018 - 2019		Total (2016 - 2019)	
		Sanctioned	Utilized	Sanctioned	Utilized	Sanctioned	Utilized	Sanctioned	Utilized
1	2	3	4	5	6	7	8	9	10
01	FIAS-100 compatible with Aanalyst 200 & Accessories	11,00,000=00	-	-	-	-	-	11,00,000=00	-
02	Tensiometer with Accessories	50,000=00	-	-	29,850=00	-	-	50,000=00	29,850=00
	TOTAL	**11,50,000=00**	-	-	**29,850=00**	-	-	**11,50,000=00**	**29,850=00**

B. Recurring

B2: Consumables:									
01	Chemicals, Kits, Outsourcing, Fertilizers & manure	1,50,000=00	15,107=00	1,50,000=00	70,545=00	1,00,000=00	-	4,00,000=00	85,652=00
02	Glassware & plastic wares	10,000=00	-	10,000=00	-	-	-	20,000=00	-
Other Items:									
B3	Travel	5,000=00	2216=00	5,000=00	21,661=00	5,000=00	-	15,000=00	23,877=00
B4	Contingency	20,000=00	-	20,000=00	10,234=00	10,000=00	-	50,000=00	10,234=00
	Total (**B2+B3+B4**)	**1,85,000=00**	**17323=00**	**1,85,000=00**	**1,02,440=00**	**1,15,000=00**	-	**4,85,000=00**	**1,19,763=00**
	Grand Total (A+B)	**13,35,000=00**	**17323=00**	**1,85,000=00**	**1,32,290=00**	**1,15,000=00**	-	**16,35,000=00**	**1,49,613=00**

Certificate

Certified that the Project work has been carried out and financial expenditure incurred for executing the Project are in accordance with the declaration / certification submitted at the time of submission of the Project Proposal and sanction obtained from time to time thereafter as per the revision made.

12. Signature of the

Principal Investigator

Vijay 13/06/18

Dr. Vijay, V.,
Scientist-B

Co-Investigator (s)

Dr. Ranjit Kar
Scientist-D

R. M...

Dr. R. Mahesh
Scientist-B

18/6/18

Mr. Gopal Chandra Das
Scientist-D

13. Signature (with comments, if any) of Head of Division

19/6/18

Sri. Debashis Chakravarty
Scientist-D
Moriculture Division

14. Signature (with comments, if any) of Director / Executive authority

see next Page

28-8-18

Director (I/c
CSR&TI-Berhampore

निदेशक / Director (I/c)
केन्द्रीय रेशम उत्पादन अनुसंधान एवं प्रशिक्षण संस्थान
Central Sericultural Research & Training Institute
केन्द्रीय रेशम बोर्ड/Central Silk Board
बहरमपुर-742101/Berhampore-742101
मुर्शिदाबाद (प.बं.)/Murshidabad (W.B.)

#	Observations of RAC	Action taken
Action: Dr. Vijay, V., Scientist-B	PI was advised to conclude the project and present the same with proper justification in the forthcoming RAC meeting.	Submitting the concluded report of the project and the same will be presented in the forthcoming RAC meeting

RAC Recommendations:

Suggestion of 48th RAC meeting dt.24.07.2018	Action taken
• The project has been concluded as the results indicated that arsenic concentration is not found to be lethal and further studies were not found to be necessary.	Complied

Directors comment:

As the study found no hazardous effect on sericulture performed using mulberry plants grown on arsenic contaminated soils (up to 21.13 mg Avl. As kg^{-1}) further study were not found to be necessary, and hence the study is concluded in midterm of the project period. Nevertheless, the study revealed the potential of mulberry plants to bio-accumulate arsenic from soil, which may be harnessed to mitigate arsenic contamination from the soils of arsenic contaminated fields.

28.8.18

DIRECTOR I/c
CSR&TI, BERHAMPORE
(Office seal)
निदेशक / Director (I/C)
केन्द्रीय रेशम उत्पादन अनुसंधान एवं प्रशिक्षण संस्थान
Central Sericultural Research & Training Institute
केन्द्रीय रेशम बोर्ड/Central Silk Board
बहरामपुर-742101/Berhampore-742101
मुर्शिदाबाद(प.बं.)/Murshidabad (W.B.)