Phytoremediation of Arsenic and Fluoride using the Mulberry-Silkworm food chain

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By

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This is to certify that the thesis entitled "Phytoremediation of Arsenic and Fluoride using the Mulberry-Silkworm food chain" is a bonafide work carried out by Sneha Mustafi under my supervision and guidance for partial fulfilment of the requirement of Master of Technology (Environmental Biotechnology) in School of Environmental Studies, during the academic session 2020-2022.

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This is to certify that sample collection and preparation for testing for the thesis entitled "Phytoremediation of Arsenic and Fluoride using the Mulberry-Silkworm food chain"; that is being submitted by Sneha Mustafi to the School of Environmental Studies, Jadavpur University Kolkata, was carried out by her under my guidance.

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This foregoing thesis is hereby is approved as a credible study of an engineering subject carried out and presented in a manner satisfactorily to its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval, the undersigned do not endorse any statement made or expressed or conclusion drawn therein bit approve the thesis only for the purpose for which it has been submitted.

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DECLARATION

This thesis work titled “Phytoremediation of Arsenic and Fluoride using the mulberry-silkworm food chain” is submitted by me, SNEHA MUSTAFI (002030904006), to the school of Environmental Studies, Jadavpur University, in partial fulfilment of the requirement for the degree of M.Tech in Environmental Biotechnology. I hereby declare that this thesis is my original work, based on the results I found, and the content of the thesis have not been submitted elsewhere for the award of any degree or any other publication. The materials of work found by other researchers and other sources are appropriately are acknowledged and mentioned by reference. I carried out my thesis work under the supervision of Dr. Subarna Bhattacharyya, Assistant Professor, School of Environmental Studies, Jadavpur University.

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Executive Summary

India is considered to be the second largest silk producer in the world. Sericulture cultivation is conducted across several states in India providing livelihood to thousands of people in the agro-based country. As silkworms are reared over several seasons in a year, it has become a promising livelihood activity among several farmers. Mulberry is a fast growing deciduous woody perennial plant with a deep root system. The plant is found extensively in tropical countries and thus, has an extensive presence in India and China. The plant is considered extremely sustainable and has a wide range of useful characteristics. The plant is considered to have several benefits including the ability to phytoremediate soils that are polluted with heavy metals. This study has been conducted to analyse bioaccumulation of arsenic and fluoride in the mulberry-silkworm food chain. Several studies have been conducted which indicate the transportation of heavy metals into the human food chain through the crops that are grown on soils that are polluted with such heavy metals. The aim of the study was to understand the amount of heavy metal that could be remediated from soils that are polluted with arsenic and fluoride. For this study, samples were collected from three locations C.S.R&T.I Berhampore, Sahebnagar, and Akalipur. Samples were collected across two rearing seasons (aghrani and falugni) to ensure the accuracy of the results. AAS method of estimation was used to identify the concentration of heavy metals in the collected samples. The results obtained, that the mulberry-silkworm food chain is much more effective for phytoremediation of arsenic contaminated soils as compared to fluoride-contaminated soils and could be used to prevent the entry of such heavy metals into the human food chain, which causes serious threats to human health. Thus, utilization of this food chain is sustainable and has the potential to prevent the entrance of such heavy metals (especially arsenic) into the human food chain and transfer into a product (silk) while creating employment for several people.

Keywords:

Phytoremediation, arsenic pollution, fluoride pollution, soil pollution, Murshidabad, Birbhum, mulberry-silkworm food chain, AAS Spectophotometry

Chapter

1. Introduction

While in the early 1900s, agriculture remained to be the main source of livelihood in India, in the 1990s, the industrial sector witnessed a rapid growth in the country. This rapid industrial growth in India, has contributed to the delivery of copious amounts of toxic effluents in the environment (Saha *et al*. 2017). The release of these pollutants both directly and indirectly into the environment has been identified to contaminate soil and water resources in the country (Saha *et al*. 2017). These toxic effluents have not only affected crop production, but have also affected the health of animals and human beings from food contamination. Current reports stated that more than 33,000 litres of wastewater and over 23,000 MLD of industrial wastewater were generated in the country in 2009, which significantly contributed towards the pollution of water resources of the country (IAS, 2019). With contaminated water sources, unregulated waste disposal systems and use of enormous amounts of pesticides, India's soil health is considered to be in a state of crisis. The fertility of the soil has been on a steep decline, which has the power to influence the food security, agricultural productivity, climate change and human health in the country. India has both big scale and small-scale industries (Saha *et al*. 2017). Small-scale industries with small numbers of effluent treatment plants or no effluent treatment plants are considered a bigger threat as compared to large-scale industries. Similarly, air borne pollutants enter the soil from emissions from different sources. Gas-dust releases into the atmosphere under high temperature technological processes, waste incineration, vehicular activities and fuel combustion; and are deposited on land surface far away from source of generation.

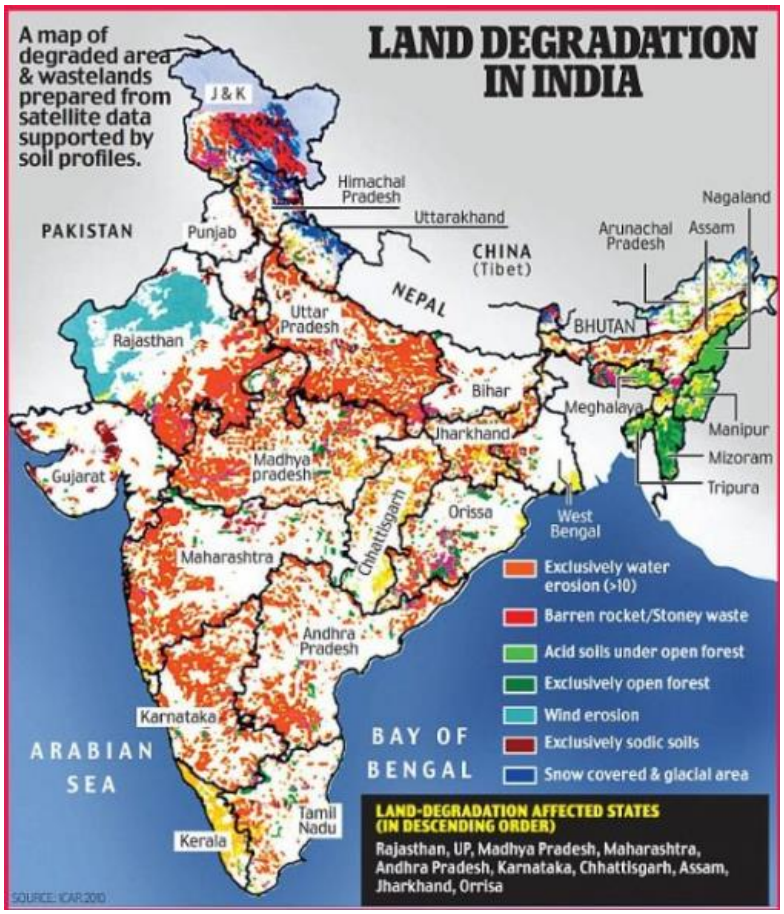


Figure 1: Soil degradation in India

Exposure to polluted soil can cause a variety of health problems in human beings. Studies carried out by various researchers indicate that soil pollution could result in food insecurity in two ways. They are - i) by reducing the crop yield due to increasing levels of toxic contaminants in the soil and ii) by making the crops that are grown in polluted soil unsafe for consumption. Consumption of contaminants through crops that are grown on polluted soil is very limited to negligible in developed countries. This is because developed countries try to maintain quality control that adheres to international standards. This however, is not the case for India where quality control is minimal and there are no specific standards set by the Indian government; or a system that overlooks the implementation of the laws that exist. Thus, the high level of soil pollution in India with a complete absence of quality control processes might result in serious health hazards among Indian citizens. To prevent any disastrous events, it is important for authorities to take initiatives, which would focus on remediation of polluted soil.

Plants play an important role in keeping Mother Nature under constant phase by reducing global warming through uptake of carbon dioxide from the atmosphere and in return produce and release oxygen into the environment; which purifies the air and provides life to animals and other organisms. Plants also contribute to soil health; retains water in soil sub-surface and cools overheated urban areas through its evapo-transpiration mechanism of water cycle (Mossad & Alazba, 2016). Ecological recycling of minerals from plants back to nature enables maintenance of fertility in soil and forests (Barot *et al.*, 2007). Beauty of plants is that; several species of plants can co-exist together in a place or unit of area (Anten, 2005) within the same environment and with the same resources (air, water, soil nutrients and predatory organisms); leading to formation of a sustainable ecosystem.

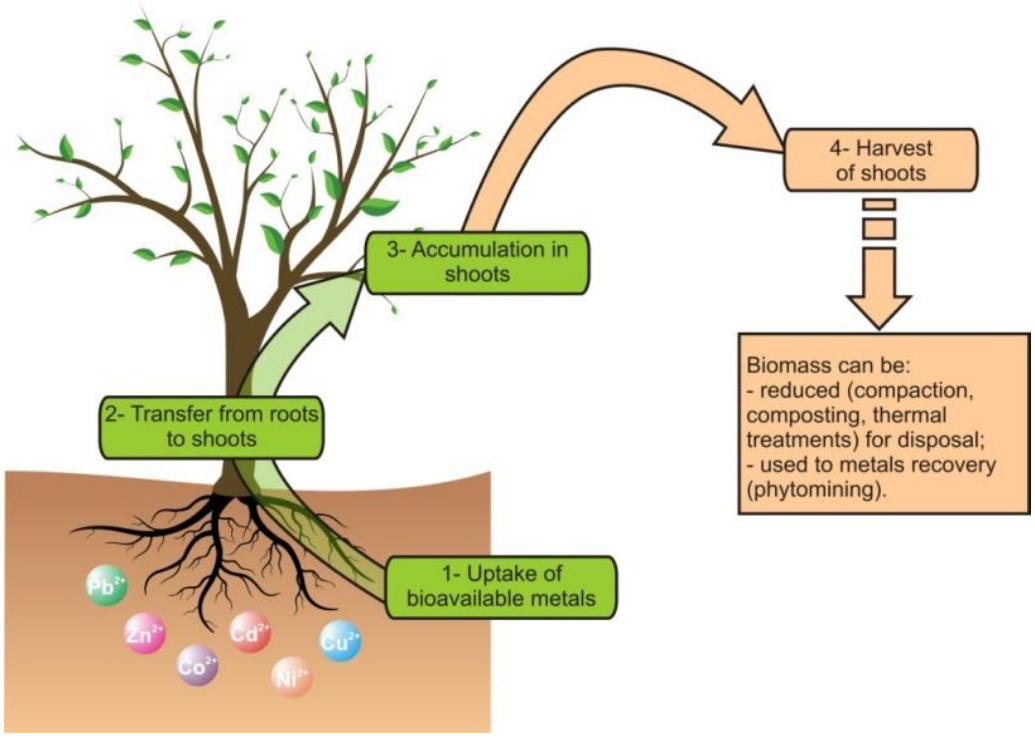


Figure 2: Phyto-remediation process

Phyto-remediation is a plant-based approach that utilizes plants to remove or extract elemental pollutants or lower their bioavailability in the soil. Plants possess the ability to absorb ionic elements in the soil (even at low concentrations) with the help of their root system (Bhawsar *et al*. 2011). Plants tend to extend their root system in the soil matrix and establish a rhizosphere ecosystem to absorb heavy metals and reduce their bioavailability in the soil, thereby reclaiming the soil and stabilizing soil fertility (Hu *et al.* 2014). Phytoremediation is widely practiced as they are economically feasible, environmentally friendly, could be applied on a large-scale, prevent erosion and further metal leaching and improve soil fertility. Various studies have been conducted over the years to understand the mechanisms underlying the heavy metal tolerance and to improve the phytoremediation efficiency of the plants (Bhawsar *et al*. 2011). Heavy metals are mostly present in the insoluble form in soil. Plants can increase their bioavailability by releasing a variety of root exudates, which can change rhizosphere pH and increase heavy metal solubility.

Mulberry is a fast growing deciduous woody perennial plant with a deep root system. The plant is found extensively in tropical countries and thus, has an extensive presence in India and China. The plant is considered extremely sustainable and has a wide range of useful characteristics (Ghosh *et al.* 2017). Some of the beneficial characteristics of mulberry involve increased foliage yield, immense environmental adaptability and shorter gestation period. In a developing country like India, mulberry is widely popular for its economic importance. The mulberry cultivation is mainly undertaken to feed and rear silkworms, with the aim of production of silk yarn (Rohela *et al*. 2020). In simple words, the protein present in the plant is converted into the silk protein (fibroin and sericin), which is the end-product of the food chain. The silk, which is thus produced, is commercially used for the production of silk garments. It has been estimated that silk obtained because of mulberry cultivation accounts for 90% of the silk production around the world (Ghosh *et al.* 2017). Thus, it could be stated that mulberry cultivation provides livelihood to many people across the globe. Moreover, the plant also offers various medicinal and nutritional benefits. Mulberry fruits and leaves are considered to have a high nutritional value and have been extensively used as animal feed or food in several countries.

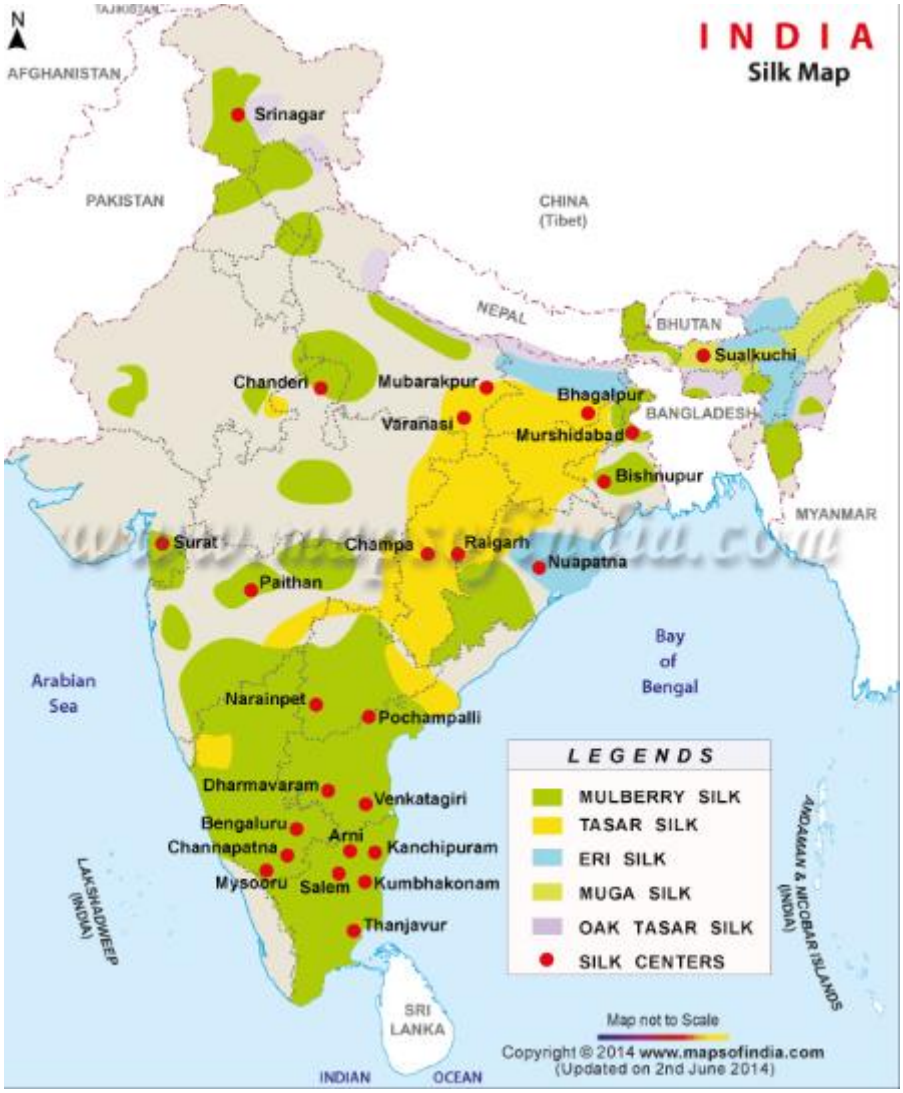


Figure 3: Silk map of India

India is considered to be the second largest producer of silk after China. There are mainly four varieties of silk that are produced in India. This includes mulberry silk, tasar silk, eri silk and muga silk. Among these four categories, mulberry silk is the major contributor (70.72%) towards India's total silk production (Arise, 2021). Thus, the characteristics that have been identified could be utilized effectively to make mulberry cultivation more sustainable in India.

Pollution has become a growing concern for government officials all across the world. Presently, increased amounts of toxic pollutants air, soil and groundwater cause serious threats to the ecology, the environment and human health. The increased amount of pollution has been attributed to deforestation, industrialization, intensive urban population and increased transportation. As a result, government bodies are in desperate need to find alternative and sustainable strategies that will help them combat pollution levels in their countries. Studies show that mulberry has been utilized in environmental protection. The salient features of mulberry like an extensive root system, strong environmental adaptability and high biomass production has provided encouraging results in stabilizing the adverse

effects of heavy metals in polluted soils. Heavy deposits of various heavy metals have been observed in the soil that arises from various anthropogenic activities. Sources of these heavy metals in soil mainly include utilization of fertilizers, mining and irrigation with contaminated water. Studies have found that woody trees like mulberry have phyto-remediation qualities. While researches have stated that the phytoremediation capability of mulberry is limited as compared to hyper-accumulators, the amount of heavy metal migration in mulberry is quite considerable due to the high biomass of mulberry. Mulberry is also considered as an effective phyto-remediation tool due to its root structure and the composition of their root exudates.

Arsenic is a highly toxic compound which poses an intense threat to a large human population. The ravine depositions in the Bengal Delta Plains have formed the fertile plains of the three rivers of Ganga, Bramhasutra and Meghna (Shrivastava *et al.* 2015). The sediment deposition in this basin mainly results from the climate and regional vegetation. As a result, the deposition of arsenic in this area is mainly controlled by the distribution of the organic matter, presence of oxic-anoxic conditions and the indigenous microbial flora. Arsenic pollution in groundwater mainly causes higher levels of arsenic in deeper levels of groundwater (Malakar *et al.* 2016). While arsenic is found in groundwater all across the world, the level of arsenic concentration is the highest in the Ganga-Brahmaputra basin. Researchers suggest that this increased levels of arsenic pollution in the groundwater is due to the increased level of tube well usage in the area. Arsenic contaminated water mainly contains arsenic acid (H_3AsO_4) and arsenous acid (H_3AsO_3) or some other arsenic derivatives (Shrivastava *et al.* 2015). Arsenic contaminated groundwater if consumed could cause serious health impacts on human beings. Moreover, being an agriculture dominant state, farmers utilize pump water to irrigate their crops. Thus, the arsenic contaminated water further contaminates the soil. Consuming crops from thos contaminated soil could also impact human health by translocating the heavy metal from the polluted soil to the human body (Handali and Rezaei 2021). Murshidabad, Malda, Bardhaman, Nadia, Hooghly and 24 Parganas are the main districts in West Bengal that face high levels of arsenic contamination in groundwater (Das *et al.* 1996).

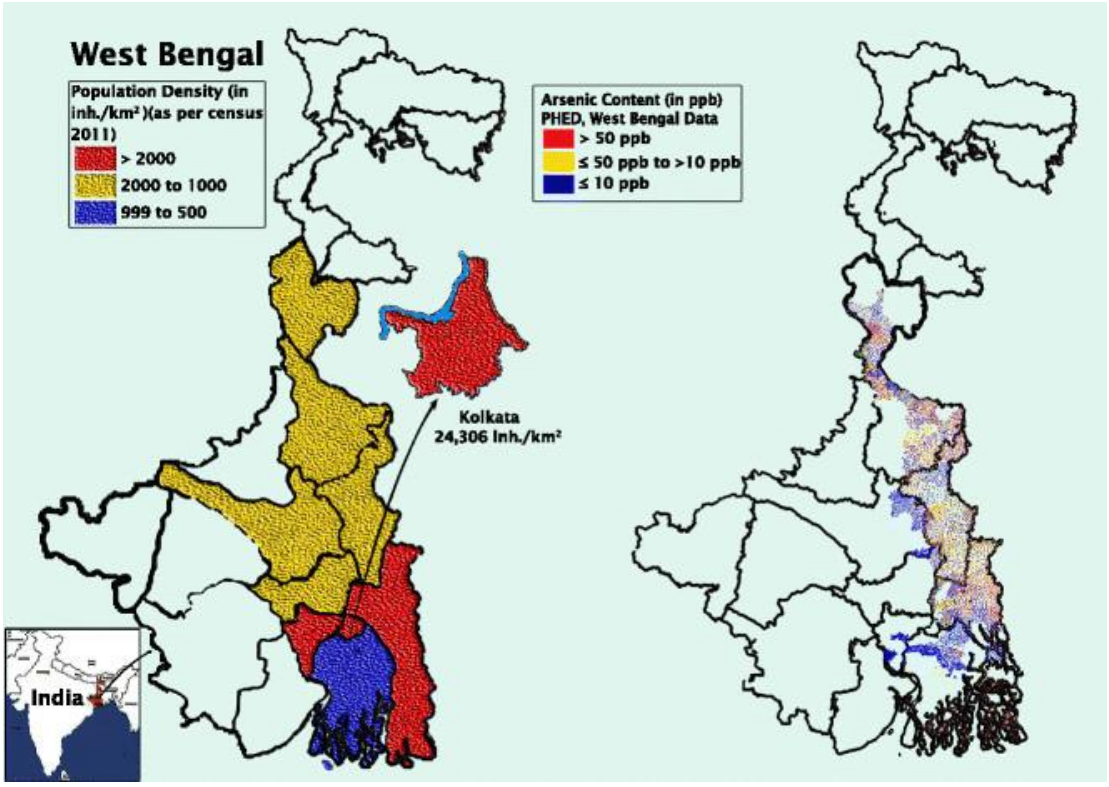


Figure 4: Arsenic contamination in West Bengal

As more than 80% of the Indian population relies on groundwater, or becomes important to ensure groundwater quality and needs to be handled separately. Higher levels of fluoride contamination have been observed all across the world, which exceeded the standards that have been provided by WHO. Higher fluoride contamination in groundwater has been mainly identified in arid and semi-arid locations. The maximum permissible fluoride limit in drinking water has been determined to be 1.5mg/L by WHO (Ali *et al*. 2019). Concentration of fluoride above 1.5mg/L either through anthropogenic and geogenic means is considered to be fluoride pollution. Fluoride contamination in groundwater has also been identified in West Bengal. The fluoride contamination has been found to be higher in the western parts of West Bengal, specifically in Birbhum, Purulia and Bankura (Ali *et al*. 2019). Recent studies have also highlighted that crops that are being irrigated with fluoride contaminated water has contributed towards an increased intake of fluoride among locals. Researchers are of the opinion that when crops are irrigated with contaminated water, fluoride gets deposited on the soil, which are then absorbed by the crops, thus entering the food chain, causing harm to the human body.

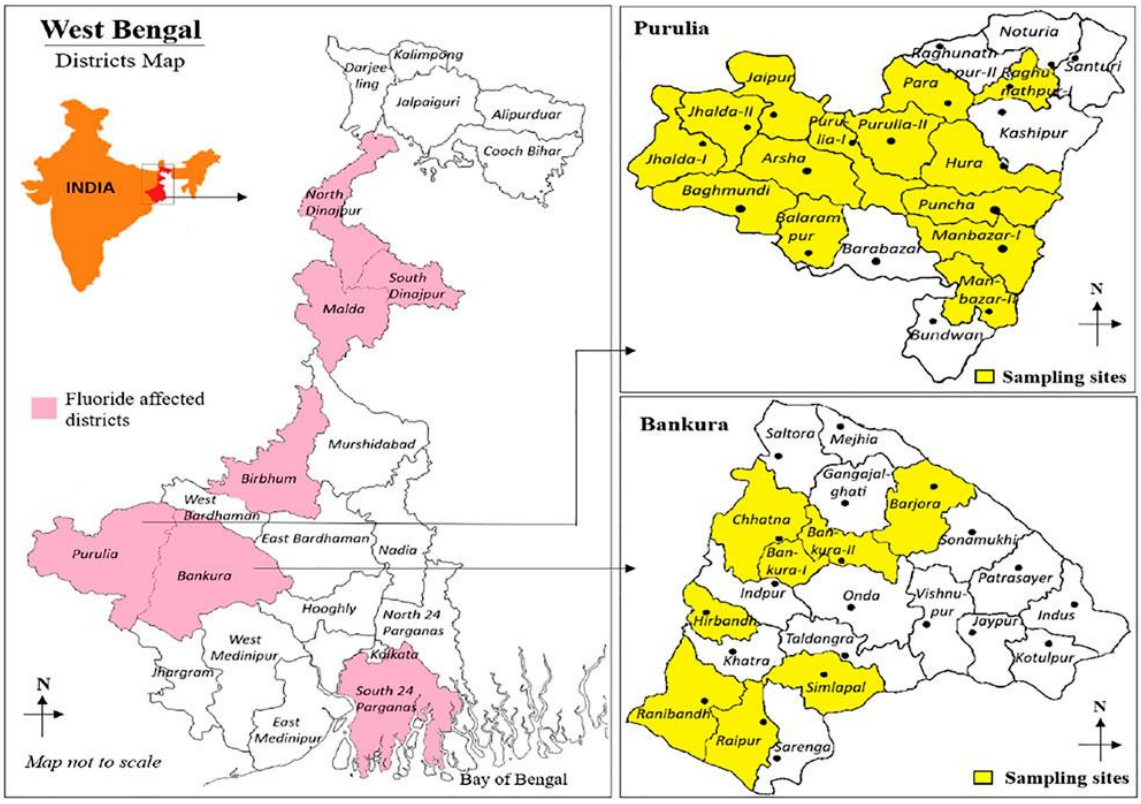


Figure 5: Fluoride contamination in West Bengal

Researchers have also examined the absorption and purification ability of some green tree species for the main air pollutants by the fumigation test, and the results indicated that mulberry is the tree species with high fluorine content (0.45 mg/g dry leaves). Later, Liu *et al.* found that mulberry has a strong retention and absorption ability to Pb and Cd by measuring the absorption and accumulation of heavy metals in the atmosphere by green tree leaves in a heavy industrial area. Studies have shown that the absorption of arsenic with the utilization of mulberry trees is more than other heavy metals such as cadmium, molybdenum, chromium and mercury (Taghizadeh & Kazemi, 2019). Studies have also shown that mulberry plants could be easily planted in soils that have low contamination levels of lead and arsenic, with concentrations lower than 369 mg/kg and 180 mg/kg respectively (Wan *et al.* 2017). The study also indicated that silkworms that were fed with mulberry leaves from trees that grew on slightly contaminated soil, exhibited positive growth and normal silk production (Wan *et al.* 2017). Mulberry plants have significant phyto-remediation qualities and have been previously used to phyto-remediate soils that were heavily contaminated with chromium. The soil in West Bengal has been found to be highly contaminated with arsenic along with lead pollution in the industrial areas. Moreover, mulberry is grown extensively in India to feed the silkworms and form an important part of the Indian silk industry. Thus, the mulberry plants could be planted in the polluted soils to remediate them and pass the heavy metals to the silk thread, which is the end-product of the silkworm food chain.

Significance of the study

It could be concluded that soil pollution is a growing problem in India. With little to no soil management strategies in the country, it is essential to identify sustainable ways to remediate polluted soils. Moreover, previous studies indicate that heavy metals have been making their way into the human food chain as a result of contaminated soils. Several researchers have indicated that crops that were grown in polluted soils transported small amounts of heavy metals into the human food chain (Zhang *et al.* 2017). While minute amounts of heavy metals might not be life threatening in the beginning, continuous exposure could result in detrimental health effects in human beings. Understanding the potential of the mulberry-silkworm food chain to bio-accumulate the heavy metals and pass them into the silk thread might be a potential solution to sustainably pass on the heavy metals into a product (silk products) and prevent them from re-entering the environment (simultaneously, the human food chain) for a significant period of time.

Chapter

2. Aims and Objectives

The primary aims and objective of this research is to identify the phytoremediation of heavy metals like arsenic and fluoride using the mulberry-silkworm food chain by using AAS (Atomic Absorption Spectrophotometry) analysis. Thus, the aims and objectives of the current study are as follows:

- To identify physiological differences between silkworms that was grown in the controlled area and the contaminated area.
- To identify levels of arsenic phytoremediation, using the mulberry-silkworm food chain
- To identify levels of fluoride phytoremediation, using the mulberry-silkworm food chain

Chapter

3. Literature Review

3.1. Soil contamination and its effects

Soil is considered to be a vital part to sustain life on earth. Soil works effectively to distribute plant and animal species and provides a habitat to a wide range of organisms. Soil however could be affected by both natural and anthropogenic activities. According to Kabata-Pendias (2000), soil quality is impacted by the various environmental factors, anthropological activities and land usage. While some changes in the soil are reversible and have effects for a very short period, some of the effects remain irreversible. Soil pollution mostly results in reducing the productivity of the soil due to the presence of soil pollutants. Soil pollutants, irrespective of the source, have an immense impact on the biological, chemical and physical properties of the soil. Fertilizers, pesticides, chemicals, discarded foods, organic manure, radioactive wastes, plastics, leather goods and industrial wastes generally cause soil pollution. In developing countries like India, where agriculture forms the major business industry, overutilization of fertilizers, pesticides and herbicides that is used to protect crops from pests, mainly causes soil pollution. The effect of such pollutants tends to be irreversible because these chemicals tend to break down or degrade very slowly (Kabata-Pendias, 2000). Simultaneously, they not only affect the growth of the plants that grow on the soil, but could also enter the food chain of human beings through the crops that are grown on the polluted soil. Thus, it could have a long lasting impact in human health. In developed countries, soil pollution might result from radioactive wastes. Radioactive wastes from industries, power plants or mining activities. Radioactive wastes could reach the soil through water or as a result of "fall-out". Das *et al.* (2004) have highlighted the fact that from the soil, crops and plants that grow in the field absorb the pollutants. Animals (livestocks), which are then consumed by humans as well often, consume these plants. Thus, it could be stated that soil pollution does not only contribute towards decreasing the fertility of the soil, but also results in moving pollutants from the soil to the human food-chain causing a wide variety of health impacts.

Soil pollution could be described as the deposition of different chemicals or heavy metals on the soil that are out of place or are in higher amounts as compared to the regular composition of the soil. According to Parr *et al.* (1992) these pollutants generally have an adverse effect on human health or other organisms. Agriculture has remained the main livelihood for communities in India. However, in the beginning of 1990s, the industrial sector began to significantly grow at a 8.4% rate (Saha *et al.* 2017). This resulted in the release of a significant amount of toxic effluents in the environment that included both land and water bodies. The release of these effluents has been identified to be directly and indirectly introducing pollutants to a vast area of water and soil resources. This has affected human health, animal health and crop production. Studies have reported that in 2009, more than 33,000 million litres of urban wastewater and more than 23,000 million litres of industrial wastewater had been generated in India. Soil degradation could be defined as the deterioration of soil quality and quantity (Saha *et al.* 2017). Soil degradation results from different factors, which include biological degradation, physical degradation, erosion and chemical degradation. Saha *et al.* (2014) have stated in their study, the majority of soil degradation of soil in India results from erosion and faulty water and soil management. It is essential to manage these resources to ensure food security of the country. Most importantly,

quality of human health is also directly linked with soil health. Thus, it is important to manage water and soil resources in areas that have industries or have a high population. Saha *et al.* (2017) have stated that small industries are more responsible for pollution of soil and water resources. This is because while large-scale industries have adequate resources to manage their effluent, small-scale industries in India, do not have the financial resources to treat their effluents before being released into the nearby land or water body. Several researchers have conducted soil health assessments extensively discreetly. The results of these assessments indicate that soil contamination with heavy metal is extremely high near industrial zones. However, many researchers are of the opinion that these results are not accurate as they do not account for the other pollution activities that are occurring in the area and less sensitive instrument technique has been utilized to obtain those results.

In India, while there are various factors that have resulted in soil pollution, geo-genically contaminated groundwater is one of the leading causes of soil pollution. A consistent rise of arsenic concentration in groundwater has been observed in several countries (Mukherjee *et al*. 2006). Similarly in India, arsenic contamination in India has been observed in several states. Saha *et al.* (2017) have stated that specifically in West Bengal high levels of arsenic contamination has been observed in groundwater and soil. According to Singh *et al.* (2011) heightened levels of arsenic contamination have been identified in areas that undertake heavy cropping activities. Higher levels of arsenic contamination have been identified in nine districts of West Bengal, mainly in the upper delta plain along the Bhagirathi river. The impact of arsenic contaminated groundwater irrigation for different crops and vegetables and their dietary intake has also been studied (Singh and Ghosh, 2011). The results indicated a significant accumulation of arsenic in different crops, which then enters into the human food chain (Zandsalimi *et al.* 2011). Fluoride contamination has also been identified in a few districts in West Bengal. Datta *et al.* (2014) has identified that bioaccumulation of fluoride is maximum in soil and could translocate in plants that are grown on contaminated soil.

3.2. Role of plants in phytoremediation

According to Yan *et al.* (2020), heavy metals that are deposited on the soil from various sources are mostly non-degradable by any type of physical or biological processes. In such cases, these heavy metals remain persistent on the soil for a long period and as a result pose a long-term threat to the environment. Heavy metals are generally categorized into essential and non-essential metals (Suman *et al.* 2018; Yan *et al.* 2020). Some of the essential heavy metals are Cu, Ni, Zn, Mn and Fe, which are essential for different biochemical and physiological processes during the plant life cycle. However, these metals can also become toxic if they are present in excess quantities (Cempel and Nikel, 2006). Some of the non-essential metals are As, Cd, F, Hg and Pb are highly toxic (Cempel and Nikel, 2006). These metals have no known importance in the plant life cycle. However, they have the potential to cause environmental pollution, adversely affect different biological and physiological processes in crops, and result in the decrease of agricultural productivity. Rehman *et al.* (2017) and Sarwar *et al.* (2010) have stated that crops that are grown in polluted soil, have the potential to translocate a portion of those heavy metals from the soil to the human food

chain. These heavy metals could accumulate in the human body through bio-magnification, which will pose a serious threat to human health.

Thus, it is important to undertake effective soil remediation measures to prevent heavy metals from entering into the atmospheric, aquatic and terrestrial environments (Gerhardt *et al.* 2009; Hasan *et al*. 2019). According to Sheoran et al. (2009) and DalCorso *et al.* (2019), there are however several methods that could be utilized to remediate contaminated soil. These processes involve isolation, vitrification, incineration, chemical oxidation and many more (Wuana and Okieimen, 2011). These processes are however often time consuming and are extremely expensive. (Ali *et al.* 2013) have reported that there are also other limitations that involve inefficiency of these processes where heavy metals are present at low concentrations; irreversible biological and physicochemical changes to the soil and might result in the overall deterioration of the soil ecosystem, with the introduction of secondary pollutants. As a result, there has been a growing need for other soil remediation approaches that would be sustainable and economically viable (Hu *et al.* 2014).

Phytoremediation could be defined as a plant-based soil remediation method (Berti and Cunningham, 2000). In this method, plants are used to extract and remove elemental pollutants or decrease their bioavailability in the soil. Yan *et al.* (2020) have stated that phytoremediation is a much more effective remediation technique as their roots are able to absorb ionic compounds even at extremely low concentrations. Jacob *et al.* (2018) have explained in their study that plants tend to extend their root system in the soil matrix and form a rhizosphere atmosphere, which is responsible for accumulating heavy metals and regulating their bioavailability in the soil. Thus, they help in reclaiming the polluted soil and stabilize soil fertility. Aken *et al.* (2010) and Jacob *et al.* (2018) are of the opinion that phytoremediation has several benefits which includes their sustainability, increases soil fertility and prevents leaching of metals and soil erosion. While soil remediation processes such as chemical oxidation introduce secondary pollutants and have the capacity to ruin the fertility of the soil, phytoremediation takes the opposite route. Plants do not introduce secondary pollutants and release organic matter, which further fertilizes the soil. Various studies have been carried out over the years to understand the mechanisms that are used by plants to remediate polluted soil and to enhance their phytoremediation efficiency.

3.3. Sericulture cultivation in India

Silk" is also known as the "queen of fabrics" and is in high demand all across the world. The fabrics produced are lightweight and soft while being durable at the same time. The leading fashion designers due to their thermo tolerance, dyeing affinity, water affinity, colours and elegance have accepted silk fabric. Gangopadhyay *et al.* (2019) is of the opinion that India produces a wide variety of silk, which ranges from Mulberry, Tussar, Eri and Muga. Geographically, Asia is considered the main producer of silk and contributes towards 95% of the worldwide silk production (Singh and Bukhari, 2022). While there are nearly 40 countries in the world that produce silk, the majority of silk is produced in China and India. China is the leading producer of silk. Bharathi (2016) has reported that in 2006, China produced around 153942 MT silk. India is the second largest producer of silk and was reported to produce around 18475 MT silk in 2006-07. India is also considered the largest consumer of

silk in the world. In India, mulberry silk is mainly produced in West Bengal, Karnataka, Tamil Nadu, Andhra Pradesh and Kashmir whereas non-mulberry silk is produced in Orissa, Jharkhand, the north-eastern states and Chhattisgarh. The silk industry involves a wide range of on-farm and off-farm activities. These activities require a diversified skill set and involve a heterogeneous group of people, bringing people of various occupations for the production of silk. Savithri and Sujathamma (2013) have stated that as mulberry cultivation is conducted over various seasons throughout the year, as a result, provide a continuous employment ro the people involved in mulberry cultivation. Sericulture is popular in India as it involves low investment with frequent income. Anitha (2011) has stated that the cultivation yields 5 to 6 crops per year once the mulberry plantation is established. Moreover, the cultivation continues to continuously yield for 15 to 16 year with a minimum expense for its maintenance.

Silk has been a way of life in India. It has obtained a prime position; and carries an aura of royalty. According to Gangopadhyay *et al.* (2019) silk has obtained a sacred place in Indian culture and is considered as a cultural heritage Silk has become an indispensable part of Indian celebrations and religious ceremonies including wedding garments for the couple. While there has been a significant development of different scales of industries, India remains an agro-based country. As a result, India still dwells in its rural parts and is highly dependent on the success of its agriculture sector. Bharathi (2016) has mentioned that simultaneously, the livelihood of more than 70% of the Indian population is dependent on the country's agricultural sector. Sericulture is an agro-based industry and is considered to be economically rewarding as it includes various sets of activities and plays a very important role in shaping the economic destiny of farmers.

Tropical climates are best suited for the cultivation of mulberry, as a result, the climatic conditions in India have been favourable and mulberry is harvested in several states across the country (Savithri and Sujathamma, 2013). The crux of the Indian silk industry predominantly lies in the states of West Bengal, Karnataka, Tamil Nadu, Jammu and Kashmir and Andhra Pradesh. These five states account for the 95% mulberry silk production in India and 95% of the total raw silk production of the country (Bharathi, 2016). Due to the growing realization of the potential of sericulture and its associated economic benefits, sericulture cultivation is gaining popularity in non-traditional locations as well. Naik (2017) is of the opinion that sericulture is best suited for a country like India because of the availability of manpower and land resources that are essential for mulberry harvesting. Moreover, sericulture has the potential to provide income to every member of the family through rearing, cocoon production, hand spinning, silk reeling and many such activities (Anitha, 2011). In the rural parts, it is often observed that female members of the family are more involved in the silkworm feeding and rearing process as they can conveniently combine their house chores with the productive work. According to recent estimates, sericulture is practiced in more than 52,000 villages and employs around 7.56 million people.

Gangopadhyay (2008) has stated that the Indian silk industry has displayed significant growth in the current years both vertically and horizontally. The schemes and plans deployed by the state and central agencies along with the relentless effort made by dedicated people in

extension and in the field of research have contributed towards the growth of the industry (Gangopadhyay, 2008). Research and development progresses like the development of mulberry varieties with higher leaf yield, new bivoltine silkworm hybrids that are more suitable for the tropical climate of the country farmer-friendly technologies and trained manpower indicates that the future prospects in the sericulture industry is likely to provide economic upliftment to financially downtrodden communities (Bharathi, 2016). However, farmers are of the opinion that the absence of any benchmark to sell their produce is hindering the sericulture industry to become popular in the farming community.

Item	Mulberry sericulture	Sugarcane	Turmeric
Total input costs	48,659	30,575	29,610
Gross returns	96,132	60,200	55,317
Net returns	47,476	29,625	25,707
CB ratio	1:1.98	1:1.97	1:1.02
Crop period	1 year	1 year	4 – 5 months

Table 1: Cost Benefit Analysis of mulberry sericulture and other comparing crops

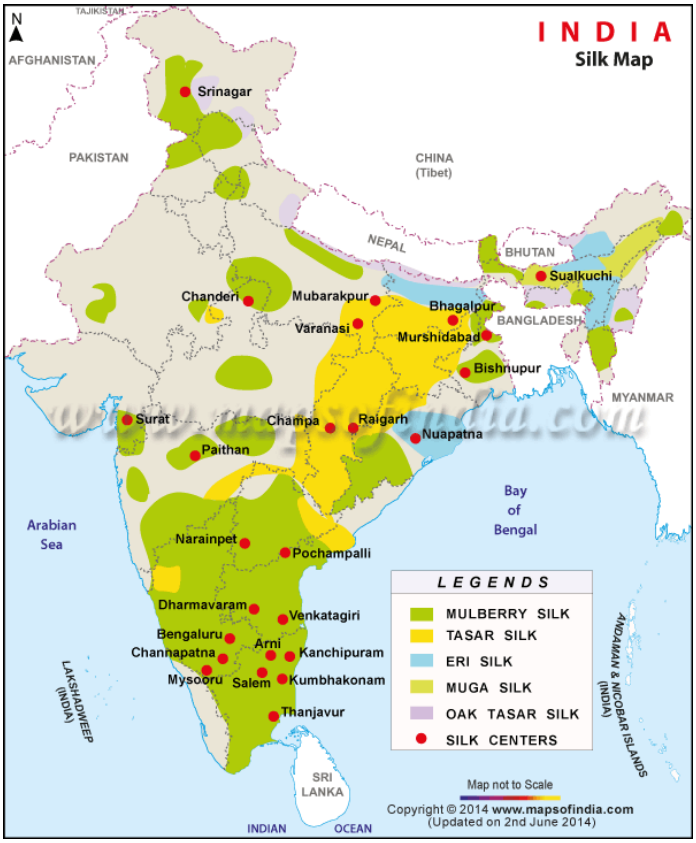


Figure 6: Silk Map of India

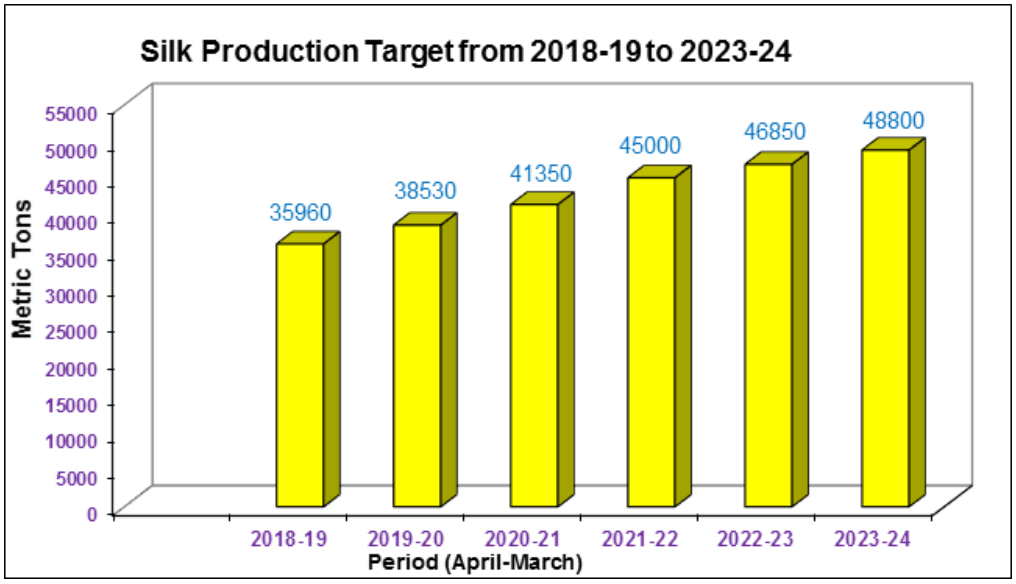


Figure 7: Silk Production Target from 2018-24

3.4. Arsenic contamination in West Bengal

Groundwater is used extensively to meet the requirements of daily purposes, which also includes agriculture. Approximately one-third of the world's population are dependent on groundwater for drinking purposes. Over the past few decades there has been a growing concern over the increasing concentration of arsenic in drinking water and has been identified

as a major public concern. Adhikary and Mandal (2017) are of the opinion that researchers have identified that a majority of the Indian population has been endangered by consuming arsenic contaminated water, where arsenic levels were significantly higher than the permitted levels. Adverse health effects of arsenic however depend on the dose and duration of the exposure (Sengupta *et al.* 2009). Intake of arsenic contaminated water with elevated levels of arsenic concentration could cause the development of arsenicosis. In India, high levels of arsenic contamination in West Bengal were first reported in 1978. In the affected regions, the arsenic concentration has been determined to exceed 50 mg/L. Santra (2017) is of the opinion that the arsenic contamination in groundwater has been growing significantly since 2006. According to surveys conducted in 2006, Murshidabad, Nadia, Malda, Hooghly, Howrah, Burdwan, North 24 Parganas and South 24 Parganas are the 8 districts that have arsenic contaminated groundwater (Santra, 2017).

Districts	Permissible Limit (BSI)	Arsenic Concentration in mg/L
South 24 Parganas	0.05mg/L	0.06-3.20
North 24 Parganas		0.06-1.28
Maldah		0.05-1.434
Nadia		0.05-1.00
Murshidabad		0.05-0.90
Burdwan		0.10-0.50
Howrah		0.09
Hooghly		0.6

Table 2: Arsenic Contamination levels in different districts in West Bengal

District	Total No. of Blocks	No. of Blocks Affected
MALDAH	15	7
MURSHIDABAD	26	19
NADIA	17	17
N.24 PARGANAS	22	19
S.24 PARGANAS	29	9
BURDWAN	31	5

Table 3: Affected blocks in different districts

The arsenic groundwater is a form of groundwater pollution, which is mainly caused due to naturally occurring arsenic in deeper levels of groundwater. The arsenic contamination that has been found in the Ganga-Brahmaputra plain is considered to be one of the major contamination zones in the world. Saha (2003) is of the opinion that the problem is mainly due to the increasing use of tube wells for water supply, which has resulted in serious arsenic poisoning in groundwater. The survey that was conducted by the Central Ground Water Board indicates that the current drainage pattern of the Ganga-Brahmaputra delta is the main cause for sedimentation in West Bengal. Acharyya *et al.* (2000) has further stated that the arsenic contamination in groundwater could have resulted from the coalfields to bring arsenic minerals from the mine to the sediments. Acharyya *et al.* (2000) mentioned that the arsenic-rich sediments that are deposited as sediments in the lower gangetic plain are brought from the Chotanagpur-Rajmahal highlands. Moreover, Boro cropping practice began in West Bengal in the late 1980s. In boro cropping, groundwater is used for irrigation, which is drawn from tube wells (Saha, 2003). This results in the decrease in groundwater levels up to (20-60 M) where the principle source of arsenic lies. The rapid decrease of the groundwater during summer seasons causes aeration, oxidizes arsenic sulphides, and makes it water-soluble; which resulted in groundwater pollution.

Fluoride becomes toxic when they are present in excess of the permissible limit (1.5ppm). Rudra (2012) has stated that excessive exposure to fluoride contaminated drinking or groundwater does not only create adverse impacts on human beings but also different animals from different species. Studies indicate that fluoride contamination is present in the western parts of West Bengal (Birbhum, Midnapore and Burdwan). Geological studies indicate that fluoride exists in the form of 'apatite', which is a naturally occurring mineral. 'Apatite' is a calcium phosphate compound. Dinajpur and Dinajpur (2011) is of the opinion that while rock-water interaction seems to be the main source of fluoride contamination in groundwater in West Bengal, the rapid decrease in groundwater levels have been attributed toward the gradual leaching of fluoride into the underground water. Moreover, prevalence of chemical and physical weathering of rocks in the arid and semi-arid conditions in high-alkaline groundwater zones further facilitate quick dissolution of fluoride in circulating water (Dinajpur and Dinajpur, 2011; Ali *et al*. 2019).

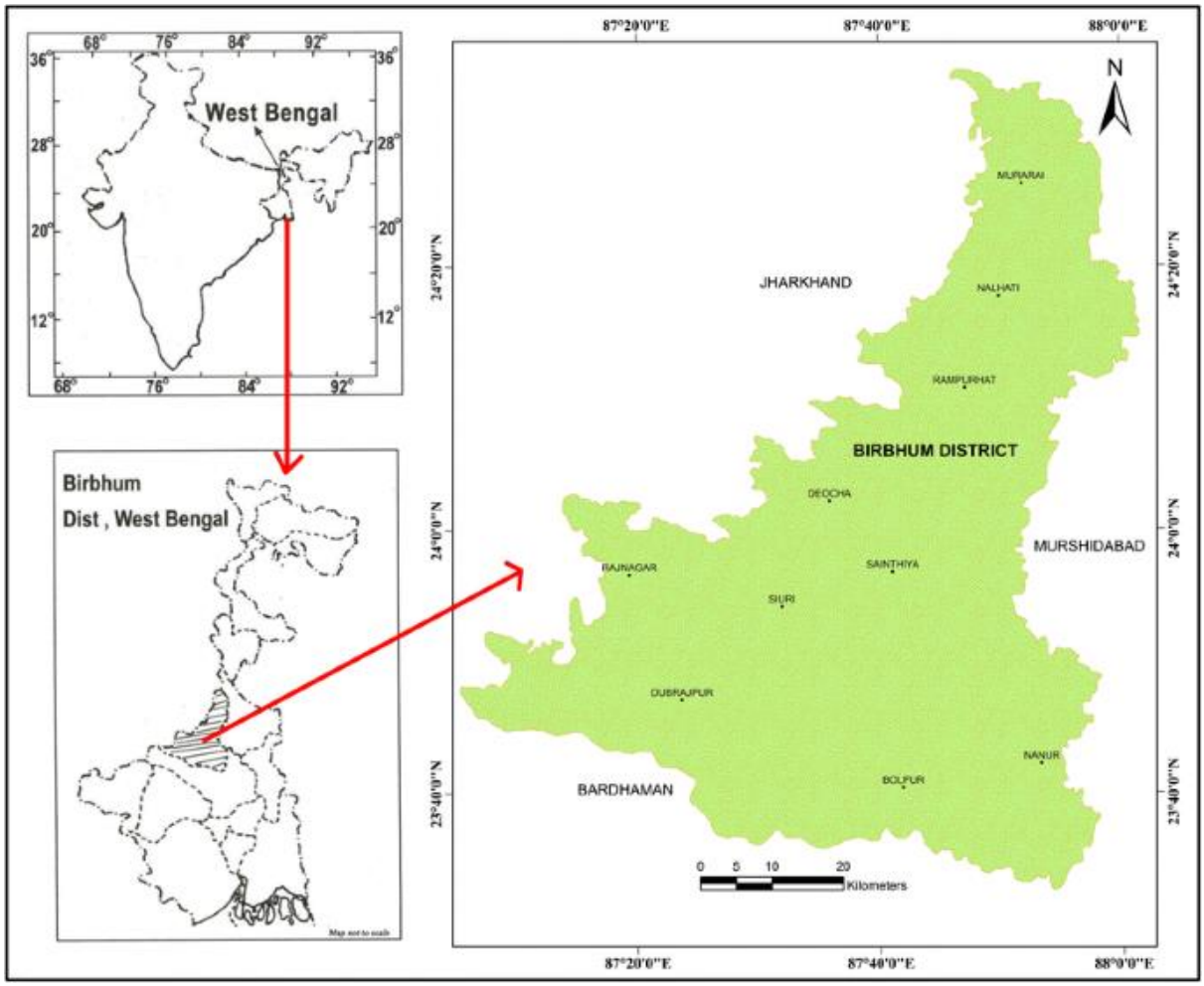


Figure 8: Fluoride affected Birbhum District, West Bengal

Both arsenic and fluoride contaminated groundwater poses direct and indirect health threats to human beings. Das (2021) has stated that consumption of crops or meat of animals that graze on grass grown on soils irrigated with contaminated water, contributes towards an increased consumption of these heavy metals in human beings. Dinajpur and Dinajpur (2011) has stated that farmers in Birbhum and Purulia extensively use groundwater for irrigation purposes. Due to lack of government intervention, this contaminated water are being used to harvest crops. The concentration of arsenic and fluoride (above permissible limits) thus becomes a factor that contributes to the accumulation of these heavy metals in agricultural soils and crops. According to Das (2021) and Bhattacharya *et al.* (2017), primarily, the contaminated groundwater is primarily used to irrigate crops and deposit a significant amount of these heavy metals on the agricultural soil. The crops that are grown on the soil, which then enters into the human food chain, then absorb these heavy metals. Thus, causing harm to human bodies.

3.5 Heavy metal phytoremediation using mulberry plants

Plants play a very vital role in restoring the environmental balance. It purifies air by taking in carbon-di-oxide and releasing oxygen (Rohela *et al.* 2020). Plants also contribute significantly towards soil health. Mother Nature possesses the ability to overcome any man-

made disturbances, provided that the harm caused is minimal. Plants play a major role in keeping the environment balanced, as they are the ones to absorb the highest quantity of pollutants that is produced due to human activities. However, very few plant species could provide environmental safety, economic generation, promotion of human health and reduction of industrial exploitation except for mulberry (Rohela *et al.* 2020). Mulberry is a fast growing woody perennial and belongs to the *Moraecae* family. The plant is well-known for its various beneficial characteristics. This involves a short gestation period, high foliage yield and strong environmental adaptability. Mulberry is known for its economic importance in producing silk through feeding of the leaf to the silkworm. The plant is also well known for its adaptation capabilities under various climates. Rohela *et al.* (2020) is of the opinion that several studies have been conducted to understand the soil remediation qualities that mulberry possesses. Jiang *et al.* 2018 and Jiang *et al.* (2020) is of the opinion that heavy metals that are present in pesticides and herbicides, degrade at a very slow rate and could pose health risk to human beings if exposed to high concentrations of those metals for a prolonged period. Ghosh *et al.* (2017) states that while soil microbes break down these heavy metals and use them as a carbon source, the process is extremely time consuming.

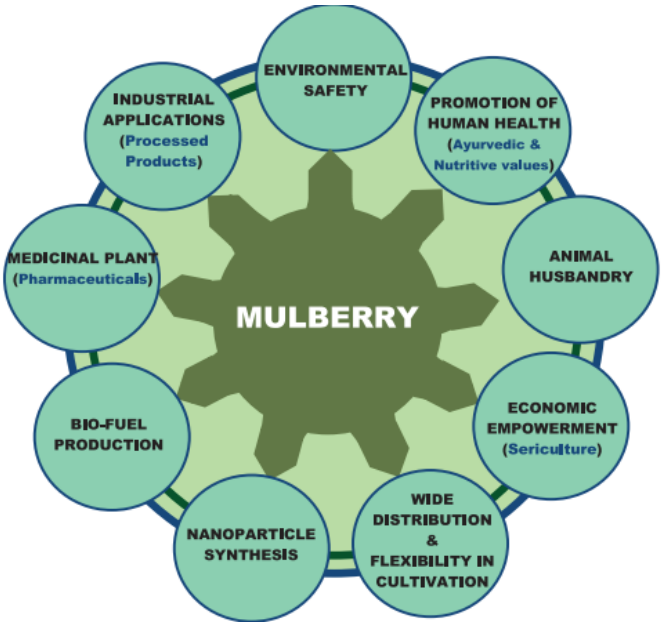


Figure 9: Role of mulberry as a multipurpose plant

Bioaccumulation of several heavy metals with the help of the mulberry plant has been studied over the years. Hashemi and Tabibian (2018) studied the accumulation of mercury (hg) in the root, stem and leaf in mulberry. For the study different concentrations of mercury nitrate were added to the soil. Their results indicated that while mercury cannot be used as a nutrient for the soil, mulberry thus reducing the excessive levels of mercury in the soil easily absorbs it. A study conducted by Ashfaq *et al.* (2012) studied the accumulation of chromium by utilizing mulberry-silkworm food chain. The results of the study indicated that a significant amount of chromium was remediated from the soil. The results indicated that while the silkworm larvae excreted a considerable amount of chromium, enormous amounts of chromium remained in the larvae's body.

Chapter

4. Methodology

4.1 Sample Collection Process

4.1.1 Materials Required

Water, soil, mulberry leaves, silkworm larvae, silkworm excreta, silk, ziplock bags, paper envelopes, paper trays, cardboard shoebox, water bottles.

4.1.2 Instruments Required

Scale, weighing machine, beakers, stirrers, hot air oven, mortar and pestle, metal sieve, measuring cylinders, volumetric flasks, pipette, funnel, AAS machine, hotplate, filter paper

4.1.3 Field study areas

For this study, three locations have been selected. They are as follows:

- Central Sericulture Research and Training Institute, Berhampore
- Sahebnagar, Murshidabad
- Akalipur, Birbhum

As the study focuses on utilization of the mulberry-silkworm food chain to remediate arsenic and fluoride from contaminated soil, it was important to select areas that have high concentrations of Arsenic and Fluoride respectively. From the previous chapters, it could be seen that while Murshidabad has high concentration of Arsenic, Birbhum has high concentration of Fluoride contamination. Thus, while being the ideal study areas for the research, these two areas also served as control locations for arsenic and fluoride contamination. This is because Birbhum has no reported arsenic contamination in the soil, while Murshidabad has no reported fluoride contamination in the soil.

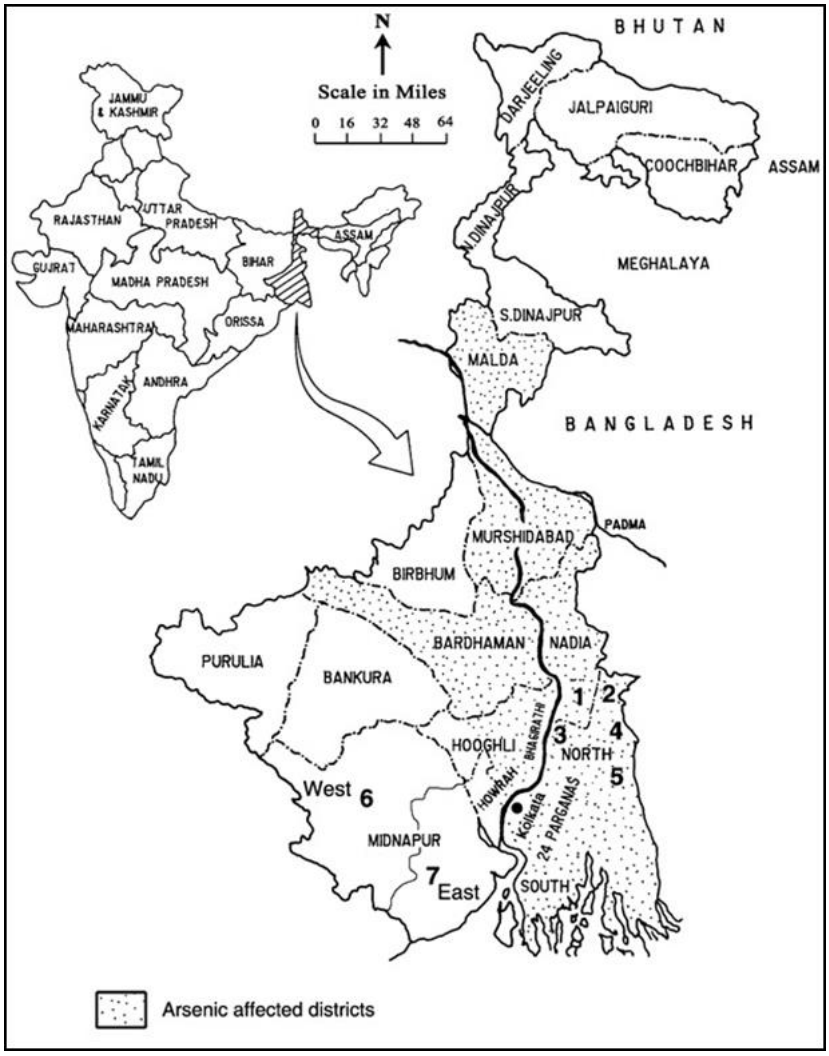


Figure 10: Arsenic affected districts in West Bengal

As the project was conducted in collaboration with Central Sericulture Training and Research Institute Murshidabad, the locations for the study were selected on the available data and convenience. As Birbhum is situated next to Murshidabad, Birbhum was selected as the control location and the study area for fluoride contamination. Field trips were arranged during the collaboration period with C.S.R&T.I in order to collect samples from Sarbanagar and Akalipur. Day long field trips were conducted in each village for several days in order to collect samples and transport them to the institute securely, while maintaining the integrity of the sample.

It was identified in the beginning stages of the study that while 5 among the six samples were easy to collect and carry, collection of silkworm larvae was extremely challenging. Silkworms are extremely sensitive organisms and could easily perish with the change of pressure and temperature. As a result, several attempts were made in order to safely bring silkworm larvae back to C.S.R&T.I so that the physical characteristics of those larvaes could be studied before preserving them for arsenic and fluoride estimation.

4.1.4 Chemicals Required

Nitric acid (HNO_3) was used for the preservation of water samples. Utilization of nitric acid mainly serves two purposes. The first objective for utilization of nitric acid is to reduce the pH of the sample below 2. Decreasing the pH level below 2 helps in reducing precipitation, adsorption to container walls and degradation of the sample through microbial activity. Secondly while utilization of AAS for analyzing heavy metal concentration sample digestion is necessary. The purpose of digestion of a sample is to break down the matrix, which could otherwise intervene during atomization. While there are several acids to choose from, nitric acid is preferred for digestion of water samples as it readily converts metal ions into their nitric salts that become highly soluble.

4.2 Collection and sampling of Soil

4.2.1 Sample Collection

Sampling procedure is a preliminary method that is carried out in a research work to develop chemical and physical data that is representative for some volume of material for a given area or time period. While grab samples have the potential to provide only a "snapshot" of the information, they play a very important role in the data collection process. In a research study, the samples that are analysed are either gran samples or composite samples. In the case of this study, the soil sample that has been utilized is a grab sample.

Grab sampling is a technique where samples are collected from one location at a single point of time. While there are other methods of sampling, this sampling method has been selected as it is both time and cost efficient. Moreover, while samples were collected from different farmers, it was observed that farmers who had cultivated mulberry in different fields were taken in order to understand the accumulation of these heavy metals in different areas. Five farmers were chosen from Sahebnagar and Akalipur at a random basis and depending on their availability. As the soil samples were collected from different farmers, it also helped to reduce the risk of any cross-contamination of the soil samples and helped in maintaining the integrity of the collected sample. In the case of C.S.R&T.I institute, samples were collected from 5 different fields within the institute campus where mulberry is cultivated. Zip lock bags were taken on sites where the samples were stored after collection and were labelled properly with the name of the farmer, location and date. Electronic weighing machine was carried to these locations in order to weigh the soil samples while they were being collected. 150gm soil was collected from each field and was secured in the sealed zip lock bags.

Figure 11: Sahebnagar Mulberry field

Figure 12: Akalipur Mulberry Field

4.2.2 Sample Preparation

The soil sample were manually spread out on paper sheets and any type of root remains, rocks and pebbles that might be mixed up in the taken soil samples were removed manually. These manually filtered soil samples were then left out in order to air dry. The air-dried sample was then passed through a sieve to remove more impurities from the collected soil samples. Following the air-drying process, the soil samples were finely grounded using mortar and pestle. These refined soil samples were then put in hot air oven at 150^0C for 5 minutes to kill bacteria that might be present in the soil sample (Hu *et al*. 2014). The soil samples were then further dried at 70^0C for 48 to 72 hours.

Figure 13: Mortar, pestle and sieve used for soil sample preparation

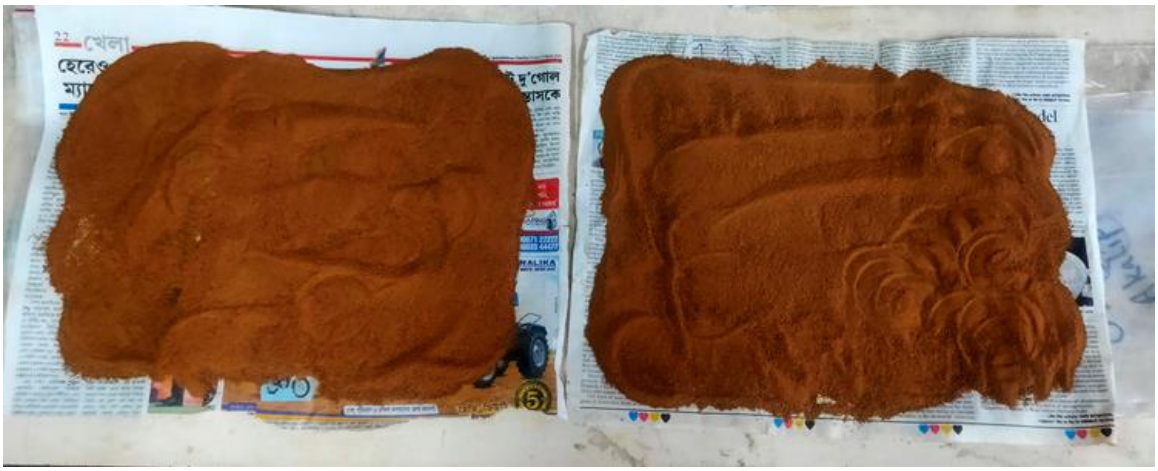


Figure 14: Prepared soil sample after drying

4.3 Collection and sampling of Water

4.3.1 Sample Collection

Grab sample could be defined as a sample that has been collected at one location at a single point of time. Another method of sample collection is known as composite collection. This is also a grab sample collection technique, which contains multiple grab samples that have been collected spanning an area at different intervals of time. Composite sampling is mainly utilized in the case of collection of samples whose composition is subject to change within a small area or over a certain period of time. For example, composite sampling is mainly used to collect wastewater as the composition of water is subject to change. However, in this study, analysis of groundwater and surface water has been undertaken that are not subject to frequent composition changes. As a result the grab sampling method has been used, as it would provide a relatively accurate depiction of the water composition spanning over a significant area.

For this study, three locations have been considered. The first one being Central Sericulture Research and Training Institute which is located in Berhampore, Murshidabad. From this location, the water sample has been collected from the field pump (groundwater) that is situated in the same field where mulberry is cultivated extensively. The second location was Sahebnagar, Murshidabad. Here too the framers predominantly use groundwater and have pumps constructed within the mulberry fields to water the plants. As a result, for Sahebnagar, water from field pumps was taken. Lastly, a water sample was collected from Birbhum, where surface water was collected from the Brahmani River. Here the cultivation fields are located adjacent to the river and as a result, farmers prefer to use the river water to cultivate their crops**.** To collect the water samples, 1-litter bottles were carried to these locations. These bottles were previously washed with distilled water to ensure the collected samples were not contaminated while being stored in the bottles. After collection of the samples, the bottles were marked (location and date of sample collection) and kept at room temperature until their testing. 2-3 drops of nitric acid was added to the water samples that were to be utilised for arsenic estimation. Addition of the nitric acid helped to prevent any bacterial growth in the water samples until the experiment is conducted.

Figure 15: Brahmani River, Birbhum

Figure 16: Sahebnagar pump water

4.4 Collection and sampling of Leaves

4.4.1 Sample Collection

Plants are considered to be a very important component in ecosystems as they transfer various elements from the abiotic environment to the biotic environment. Several researches have indicated that plant leaves tend to have high amounts of heavy metals because of bioaccumulation. As the main aim of the study is to analyse phyto-remediation of arsenic and fluoride using mulberry-silkworm food chain, it is essential to understand the amount of heavy metals that gets stored in the leaves. This helps to understand the amount of heavy metals that gets deposited at one of the most crucial parts that is involved in the mulberry-silkworm food chain.

The sampling of leaves was conducted two times. The first sampling of mulberry leaves was conducted in December 2021 and the second sampling was conducted in March 2022 (Falguni crop). The leaf samples were taken from three locations for both times that they were collected. The control samples were collected from all three locations. The control samples for arsenic estimation were collected from Akalipur, Birbhum whereas the control samples for fluoride estimation were collected from C.S.R&T.I, Murshidabad and Sahebnagar, Nabagram. The S1-1635 variety of mulberry plant has been considered for the study. S-1635 is a high yielding mulberry variety that is mostly popular for Eastern and North-eastern regions (Chakraborty *et al*. 2020). The S-1635 is mainly recommended for both rain fed and irrigated conditions. Straight branches characterize this mulberry plant variety, erect growth, large thick dark green leaves which have high moisture content. Other

advantages of the S-1635 leaf variety include their quick regeneration capacity with high vigour, good propagation, have high rooting ability and are suitable for irrigated conditions. Fifteen (15) leaves were collected from five farmers of each village whereas 15 leaves were collected from different fields within the institute itself.

4.4.2 Sample Preparation

As the plant variety that had been chosen for sample collection are small trees that grow in bush plantations, they were potentially exposed to soil splash on their leaves. The leaves were collected and were put in paper envelopes. In order to prevent cross-contamination; the leaf sample was put through a three-step washing sequence. Firstly, the leaves were rinsed with 0.1% Teepol, followed by rinsing them with 0.1% HCl and then rinsing them with distilled water (Hu *et al*. 2014). Following this process, the leaves were air-dried. Following the air-drying process, the leaves were killed in the hot air oven for 5 minutes at 105^0C and then dried for 18 to 72 hours at 70^0C, till a consistent weight was achieved. The dried leaves were then weighed and manually grounded using the mortar and pestle, to prepare them for the digestion process.

Figure 17: S-1635 Mulberry Leaves

Figure 18: Dried Mulberry Leaves

4.5 Collection and Sampling of Silkworm

4.5.1 Sample Collection

Various silkworm breeds are reared for the production of silk. Collection of silkworm was an important part of the study as it was essential to analyze the bioaccumulation of heavy metals in silkworms as they are the main component responsible for completely transporting these heavy metals from the human food chain to the mulberry silkworm food chain. Bivoltine (N x SK6 x SK7) and multivoltine (N x M 12 (W)) hybrids are the main silkworm breeds that are currently used both by the sericulture institute and the farmers for the production of silk (Chakraborty *et al*. 2020).

For this study, the Bivoltine hybrid (N x SK6 x SK7) hybrid was utilized. The silkworm larvae were reared by feeding S-1635 mulberry leaves under irrigated conditions. The silkworm larvae samples that were collected for the study were fed four times a day (6 am, 11 am, 4 pm and 8pm). Silkworms are very sensitive creatures and need to be fed at regular intervals in order to sustain their healthy growth and ensure good quality silk production. Silkworm larvae were collected over two crop seasons to increase the accuracy of results obtained for the study. The November crop (Aghrani) and February crop (Falguni) were considered for the study and the silkworm samples were collected during these two seasons to ensure accuracy of the data obtained across different rearing seasons. 25 silkworm was collected from 5 farmers in each location. The average temperature and humidity during the November crop was recorded to be 18.5^0C and 53% respectively. The average temperature and humidity during the February crop was recorded to be 23^0C and 58% respectively.

The collection process of silkworm larvae was very difficult in the first few attempts. Research indicates that various factors play a crucial role in maintaining the growth and productivity of silkworms. Temperature is one of the abiotic factors that are of vital importance. Studies also indicate that silkworm at the later stages (5^{th} stage) prefers lower temperatures as compared to silkworm larvae at younger stages. Silkworms are poikilothermic, and due to their high sensitivity to temperature, transporting silkworms to the institution became a challenge as most larvae died as they were transported back to C.S.R & T.I. At first, for the collection process, zip lock bags a big plastic container was used to store the larvae. However, this collection process was not effective and the larvae died during the first three collection attempts. After consultation with the farmers, a cardboard shoebox was utilized as the storage container. Several perforations were made in the cardboard box to ensure that the larvae could breath and the temperature inside of the cardboard box remained low. This process was successful and helped to bring silkworm larvae to the institute in living condition. The samples were taken in small plastic bags for which the mouths were kept open to facilitate the larvae to breath. Weight of 10 larvae were taken on the field to compare them to the ideal weight that healthy silkworm larvae must have.

4.5.2 Sample preparation

It was essential to start the sampling process at the earliest as larvae at the 5^{th} stage tend to form cocoons within a very short span of time. Paper trays were made in which silkworm larvae samples were placed and marked with the location and name of the farmers from

whom the silkworm larvae were collected. The silkworm larvae were killed in the hot air oven at 105^0C for 5 hours and then it was dried at 70^0C for 48 to 72 hours.

Figure 19: Silkworm larvae collection, Birbhum

Figure 20: Silkworm Larvae Collection, C.S.R & T.I

Figure 21: Silkworm larvae collection, Sahebnagar

Figure 22: Silkworm larvae collection, Akalipur

Figure 23: Silkworm larvae collection, Akalipur

Figure 24: Perforated cardboard box used for effective collection and storage of silkworm larvae

Figure 25: Dried silkworm larvae on paper trays

4.6 Collection and Sampling of Excreta

4.6.1 Sample Collection

Excreta of silkworms were collected in order to estimate the amount of heavy metals that were discarded during the entire lifetime of a silkworm larva. This helped to understand the total amount of heavy metals that is getting remediated through the mulberry-silkworm food chain. Excreta were collected from the same farmers from whom the silkworm larvae had been collected for the study. The excreta was collected from three locations (Murshidabad, C.S.R & T. I, Birbhum). The excreta samples were collected from five farmers that provided the silkworm larvae for the study. The excreta were collected in zip lock bags. Approximately 50 grams of excreta was collected from each farmer. Those zip lock bags were then sealed and marked.

4.6.2 Sample Preparation

The excreta samples were then transferred into paper trays that were put in the hot air oven for the drying process. The excreta samples were then dried in the hot air oven at 70^0C for 48 to 72 hours.

4.7 Collection of Silk

4.7.1 Sample Collection

The silk collection is the last stage of the collection process. Silk is the final product that is produced at the end of the food chain and is hence destined to store the final amount of heavy metal that gets remediated from the soil. For the silk collection process, Cocoons were collected from which the silk threads were extracted. Cocoons of silkworm larvae from the bivoltine breed were selected to maintain the uniformity in the study. Cocoons were collected from three locations (Murshidabad, C.S.R & T. I and Birbhum) for this study. Cocoons were collected from the same five farmers that have participated throughout the study. A minimum of 100 cocoons were collected from the farmers from which the silk threads were to be

extracted. The cocoons were collected in zip lock bags. These bags were then marked and sealed.

4.7.2 Sample Preparation

The cocoons were then transferred in paper trays and placed in the hot air oven for the drying process. The cocoons were required to be subjected to high heat at the beginning to kill the larva that was present inside the cocoon. This ensured that the shell remained intact and unbroken silk threads could be obtained at the end of the drying process. The larvae within the collected cocoons were killed by subjecting them at 105^0C for 5 hours. Once the larvae within the cocoon were killed, the cocoons were dried at 70^0C for 48 to 72 hours.

After the completion of the drying process, the cocoons were dissolved in boiling water to facilitate the extraction of individual long silk fibres to be extracted. This helps to remove the gum (sericin) that is present on the thread and holds the cocoon together, thus making the extraction process easier. This process is also important as it ensures that there is no damage to the continuity of the silk thread. A small container was placed on a small stove. Once water in the container started to boil, the cocoons were dissolved in the boiling water in batches. Once the gum was removed from the cocoon, the silk threads were obtained by winding them on a reeling machine. The obtained silk threads were tied in a knot and were placed on a paper tray for the drying process. The silk threads were then dried in the hot air oven at 70^0C at 48 to 72 hours.

Figure 26: Gum removal from cocoon

Figure 27: Cocoon reeling (thread extraction)

Figure 28: Cocoon reeling (thread extraction)

4.8 Physical Characteristic Examination

The physical characteristics of the silkworm larvae were analysed to identify any visible difference between the samples that had been collected from the control and heavy metal fortified areas. The length and weight of the silkworm larvae was studied to understand the morphological changes that had been imposed by the heavy metal accumulation. As silkworm larvae are very delicate creatures, it was important to ensure that the larvae were alive until the physical characteristics have been measured.

Weights of the collected larvae were measured in the respective locations with the help of electronic weighing machines. Weight of one larva was taken followed by taking down the weight of ten (10) larvae. This was conducted in order to compare the weight of 10 silkworm larvae to the ideal weight of healthy silkworm larvae. The obtained weights were written down to analyse any difference between the silkworm that has been collected from areas where the soil is fortified with Arsenic and areas that were polluted with fluoride. After the successful transportation of silkworm larvae in the sericulture institute, the length of each larvae was measured. The obtained lengths were written down to analyse any difference between the silkworm that has been collected from areas where the soil is fortified with Arsenic and areas that were polluted with fluoride. Si *et al.* (2021) suggests that various heavy metals tend to have different effects on silkworms that grow on soil that are fortified with heavy metals. Analysing the physical characteristics has not only helped to understand the impact of arsenic and fluoride on the morphology of silkworm larvae, but has also helped to compare the morphological changes between larvae that grew in arsenic and fluoride contaminated areas.

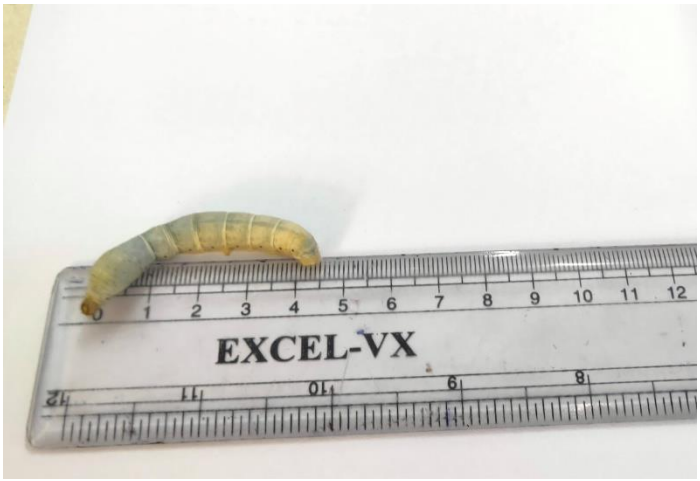


Figure 29: Measuring length of silkworm larvae

Figure 30: Weighing silkworm larvae

Figure 31: Weighing silkworm larvae

4.8 Estimation of Arsenic

4.8.2 Arsenic analysis in soil sample

The collected soil samples were oven dried and finely powdered. 0.5 gram of homogenized soil samples were digested with 10 ml of a 3:1 HCL/HNO_3 mixture in a kjeldahl digestion tube. The digestion tubes were left overnight at room temperatures and then placed in a heating block. Each tube was covered with an air condenser and refluxed gently for 2 hours at 80^0C (Zandsalimi *et al*. 2011). Whitman No. 40 filter papers were moistened and were prepared for the filtration process. The samples were then cooled down, the digests were then filtered with the use of Whitman filter no. 40 into a 50 ml volumetric flask and 10 ml of the solution containing 10% HCl, 5% ascorbic acid and 10% KI was added (Zandsalimi *et al*. 2011). Flasks were then made up to volume with distilled water. Arsenic (As) was performed with the use of atomic absorption spectrophotometry.

4.8.3 Arsenic sample in plant sample

The plant samples were cleaned using a three-step washing sequence. Firstly, the leaves were washed with 0.1% Teepol, followed by rinsing them with 0.1% HCl and then rinsing them with distilled water (Hu *et al*. 2014). Following this process, the leaves were air-dried. Following the air-drying process, the leaves were killed in the hot air oven for 5 minutes at 105^0C and then dried for 18 to 72 hours at 70^0C, until a consistent weight was achieved. The over-dried leaf samples were in a stainless steel mill to obtain a homogeneous sample and prepared for digestion. 0.5 gram of ground samples were placed in a digestion tube and mixed with 2.5 ml of concentrated nitric acid. The digest was allowed to stand overnight and then 2.5ml of H_20_2 was added (Zandsalimi *et al*. 2011). The tubes were placed in a digestion block and heated to 100^0C, until frothing ceased then heated at 140^0C until the solution became clear. The tubes were then heated to 180^0C to boil off the nitric acid. On cooling, the residue was taken up in 10ml of a solution containing 10% HCl, 5% ascorbic acid and 10% KI (Zandsalimi *et al*. 2011). Arsenic concentrations were measured in duplicate using an atomic absorption spectrophotometer.

4.8.4 Arsenic analysis in organic matter

The over-dried larvae, excreta and silk samples were in a stainless steel mill to obtain a homogeneous sample and prepared for digestion. 0.5 gram of ground samples were placed in a digestion tube and mixed with 2.5 ml of concentrated nitric acid. The digest was allowed to stand overnight and then 2.5ml of H_2O_2 was added. The tubes were placed in a digestion block and heated to 100^0C until frothing ceased then heated at 140^0C until the solution became clear (Zandsalimi *et al*. 2011). The tubes were then heated to 180^0C to boil off the nitric acid. On cooling, the residue was taken up in 10ml of a solution containing 10% HCl, 5% ascorbic acid and 10% KI. Arsenic concentrations were measured in duplicate using an atomic absorption spectrophotometer.

4.9 Estimation of Fluoride

4.9.1 Fluoride analysis in water

The collected water samples were stored in glass containers. After shaking the containers, 50ml of water samples were marked to prevent any confusion or contamination. A series of dilutions were prepared by diluting various volumes of standard fluorine solutions to 100 mL in tubes. The range has been restricted between 0 to 1.4 mg/L. to each 50ml standard solution, 10 mL of acid-zirconyl-alizarin reagent was mixed. The wavelength of spectrophotometer was set at 540 nm. The spectrophotometer was set to zero absorbance with the reference solution that is distilled water with reagent. The solution was placed in the spectrophotometer to read the absorbance.

4.9.2 Fluoride analysis in soil

Soil samples were dried and sieved to obtain homogenized samples for the study. 1.05gram of soil samples (from three locations) were digested with aqua regia. The digestion of the soil samples were completed on a pre-heated hot plate for about 15 to 30 minutes until a clear coloured solution was obtained. The digested sample was left to cool down. The digested cooled sample was filtered into a 250ml volumetric flask through a whatman no. 4 filter paper (Imran and Donald, 2021). A blank serve as a control was prepared using the same procedure except without any sample. All solutions were analysed for fluoride using a Spectrophotometer and SPADNS (Sodium 2-(parasulfophenylazo)-1, 8-di-hydroxy-3, 6-naphthalenedisulfonate) reagent at 580 nm wavelength (APHA. 2005). The reagent is made up of Acid-zirconyl-SPADNS reagent: a mixture of equal volumes of SPADNS solution (5.5) and zirconylacid reagent (5.6). For calcium determination, 1 ml of sample was added to 99 ml of distilled water, followed by 2 ml of NaOH was added to the resultant solution. One to two drops of murexide indicator was added to the resultant solution. All this content was titrated against EDTA.

4.9.3 Fluoride analysis in plant leaves

The plant samples were cleaned using a three-step washing sequence. Firstly, the leaves were washed with 0.1% Teepol, followed by rinsing them with 0.1% HCl and then rinsing them with distilled water (Hu *et al*. 2014). Following this process, the leaves were air-dried. Following the air-drying process, the leaves were killed in the hot air oven for 5 minutes at 105^0C and then dried for 18 to 72 hours at 70^0C, till a consistent weight was achieved. The samples were grounded and prepared for the fluoride estimation process. 100 grams of the over-dried leaves were grounded and passed through a mesh sieve. Any unsieved materials were sealed and kept aside for future use. About 0.5 g each of the powdered samples was transferred into a 150-mL nickel crucible and moistened with a small amount of de-ionized water. 6ml of 16.8 N NaOH was added and the crucible was placed in an oven (150°C) for 1.5-2.0 hr until NaOH was solidified. The crucible was placed in a muffle furnace set at 300°C, then raised to 600°C and kept at 600°C for 30 min in order to fuse the sample in the crucible. The crucible was placed in a hood and allowed to cool, and 10 mL distilled water was added. Then, 37% HCl solution (about 7 mL) was added slowly to adjust the pH to 7-9. The sample solution was transferred to a 100 mL plastic volumetric flask, made up to volume

with distilled water and filtered through a Whatman No. 40 filter paper. A 25ml aliquot of vegetable was mixed with 25ml TISAB II, used for analysis of fluoride with the same procedure and the same ion selective electrode used for water analysis.

4.9.4 Fluoride analysis in organic matter

The samples were grounded and prepared for the fluoride estimation process. 100 grams of the over-dried leaves were grounded and passed through a mesh sieve. Any unsieved materials were sealed and kept aside for future use. About 0.5 g each of the powdered samples was transferred into a 150-mL nickel crucible and moistened with a small amount of de-ionized water. 6ml of 16.8 N NaOH was added and the crucible was placed in an oven (150°C) for 1.5-2.0 hr. until NaOH was solidified. The crucible was placed in a muffle furnace set at 300°C, then raised to 600°C and kept at 600°C for 30 min in order to fuse the sample in the crucible. The crucible was placed in a hood and allowed to cool, and 10 mL distilled water was added. Then, 37% HCl solution (about 7 mL) was added slowly to adjust the pH to 7-9. The sample solution was transferred to a 100 mL plastic volumetric flask, made up to volume with distilled water and filtered through a Whatman No. 40 filter paper. A 25ml aliquot of vegetable was mixed with 25ml TISAB II, used for analysis of fluoride with the same procedure and the same ion selective electrode used for water analysis.

4.10. Bioaccumulation Factor

Bioaccumulation could be described as the increment of contaminant concentrations in organisms following its uptake from the ambient environmental medium. The bioaccumulation factor was calculated for both arsenic and fluoride. The bioaccumulation factor is measured to understand the bioaccumulation of heavy metals in different samples that has been collected during the study.

$$\textbf{Bioaccumulation Factor (BAF)} = \frac{\textbf{Concentration of tissue}}{\textbf{Concentration in soil}}$$

Chapter

5. Results and Discussion

5.1. Physical characteristics of silkworm

Apart from understanding the effectiveness of the mulberry-silkworm food chain to remove heavy metals (Arsenic and Fluorine), the physical characteristics of silkworms were analyzed to identify any similarities between the data of silkworms collected from the control area and the ones that were collected from the contaminated area.

5.1.1. Weight of silkworm

Silkworm samples were collected from three different locations that are C.S.R&T.I Berhampore, Sahebnagar (Murshidabad) and Akalipur (Birbhum). Weights were measured directly after the collection of the samples in order to ensure that the accurate measurement was taken (no bodily fluid is lost due to silkworm death). Single as well as the collective (weight of 10 silkworms) weight of silkworms were taken for each sample collected from these three locations. The weight of the collected samples has been listed down below:

Table 4: Weight of silkworm larvae collected from C.S.R&T.I Berhampore

C.S.R&T.I Berhampore		
Sample No.	**Weight of single larva, aghrani crop (gm)**	**Weight of single larvae, falguni crop (gm)**
R1	3.12	2.67
R2	3.22	2.62
R3	2.80	2.73
R4	2.72	2.56
R5	2.42	2.52
R6	2.12	2.57
R7	2.34	2.28
R8	2.56	2.41
R9	2.40	2.30
R10	2.35	2.33
Weight of 10 larvae (gm)	**26.05**	**24.99**
Avg. Weight	**2.60±0.35**	**2.49±0.16**

Table 5: Weight of silkworm larvae collected from Sahebnagar, Murshidabad

Sahebnagar, Murshidabad		
Sample No.	**Weight of single larva, aghrani crop (gm)**	**Weight of single larvae, falguni crop (gm)**
R1	2.12	2.35
R2	2.44	2.26
R3	2.42	2.17
R4	2.50	2.24
R5	2.73	2.38
R6	2.65	2.45
R7	2.83	2.36
R8	2.63	2.67
R9	2.14	2.30
R10	2.20	2.15
Weight of 10 larvae (gm)	**24.66**	**23.29**
Avg. Weight	**2.46±0.25**	**2.33±0.15**

Table 6: Weight of silkworm larvae collected from Akalipur, Birbhum

Akalipur, Birbhum		
Sample No.	**Weight of single larva, aghrani crop (gm)**	**Weight of single larvae, falguni crop (gm)**
R1	3.01	3.26
R2	2.59	3.14
R3	2.78	3.18
R4	3.25	3.42
R5	2.61	3.20
R6	2.74	2.86

R7	3.20	2.88
R8	3.15	3.12
R9	2.85	2.59
R10	2.92	2.74
Weight of 10 larvae (gm)	**29.10**	**30.42**
Avg. Weight	**2.91±0.23**	**3.09±0.25**

Chart no. 1: Average weight of silkworm larvae among three locations (aghrani crop)

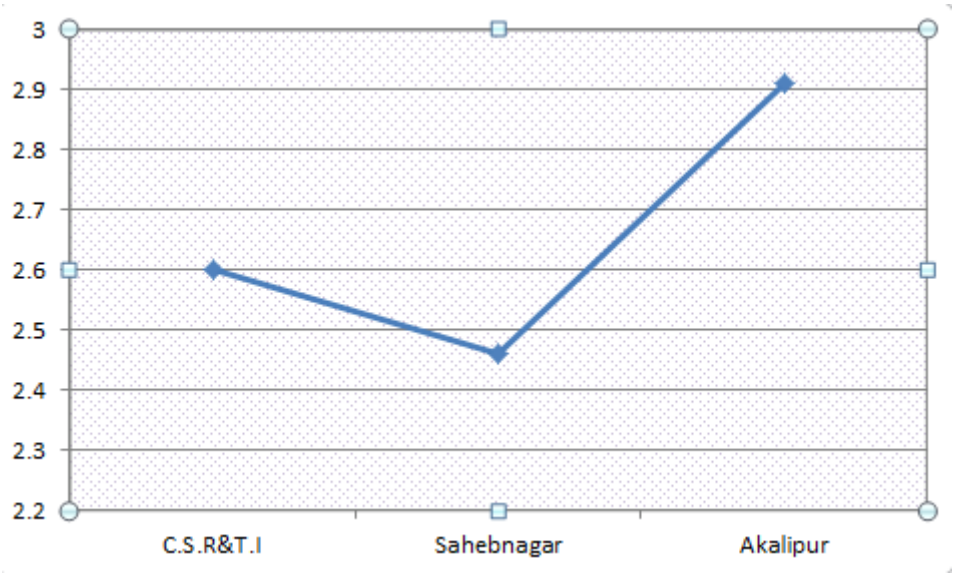
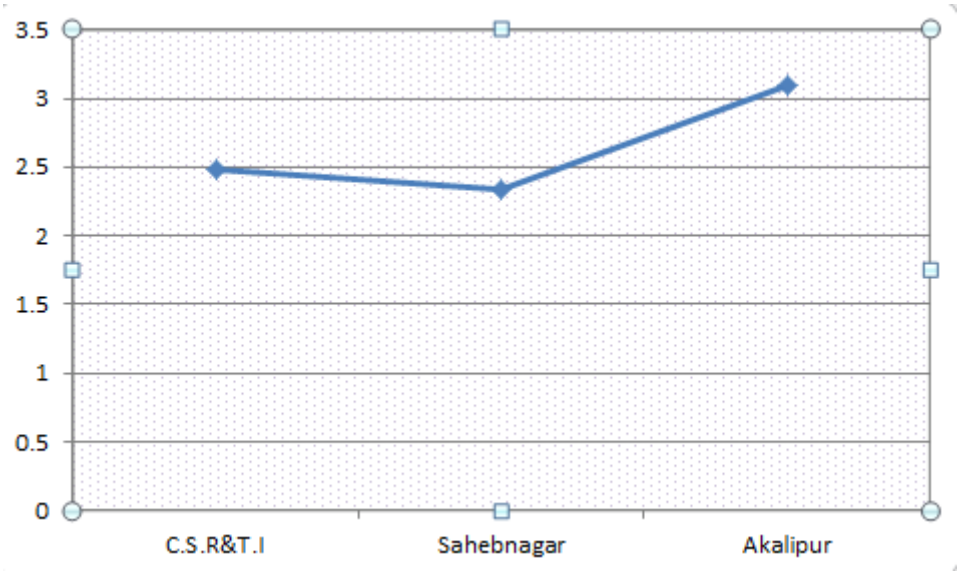


Chart no. 2: Average weight of silkworm larvae among three locations (Falguni crop)



From the data obtained from the above table and graph, it could be concluded that the average weight of silkworm larvae collected from fluoride contaminated region (Akalipur) is significantly higher as compared to the larvae samples collected from arsenic contaminated areas (C.S.R&T.I and Sahebnagar). This finding could be supported by the findings that have been observed by Handali and Rezaei. Handali and Rezaei (2021) have stated in their study that arsenic consumption has been contributed towards weight loss in organisms. The researchers have stated that arsenic interferes with the endocrine system, leptin and adiponectin hormones as well as thermogenesis (Handali and Rezaei, 2021). The ideal average weight of 10 larvae is 36gm. Thus, while silkworm larvae that grew in fluoride contaminated soil were affected, most impact on the average weight could be observed more evidently in the samples that were collected from the arsenic contaminated regions.

5.1.2. Length of silkworm larvae

Silkworm samples were collected from three different locations that are C.S.R&T.I Berhampore, Sahebnagar (Murshidabad) and Akalipur (Birbhum). Length was measured directly after the collection of the samples in order to ensure that the accurate measurement was taken (no bodily fluid is lost due to silkworm death). The length of the collected samples has been listed down below:

Table 7: Length of silkworm larvae collected from C.S.R&T.I, Berhampore

C.S.R&T.I, Murshidabad		
Sample No.	**Length of single larva, aghrani crop (cm)**	**Length of single larvae, falguni crop (cm)**
R1	5.5	6.0
R2	6.1	5.5
R3	5.5	5.6
R4	5.8	5.7
R5	6.0	5.9
R6	6.2	5.2
R7	5.4	5.4
R8	5.6	5.2
R9	5.3	6.2
R10	5.7	5.7
Avg. length	**5.71±0.30**	**5.64±0.33**

Table 8: Length of silkworm larvae collected from Sahebnagar, Murshidabad

Sahebnagar, Murshidabad		
Sample No.	**Length of single larva, aghrani crop (cm)**	**Length of single larvae, falguni crop (cm)**
R1	6.0	6.0
R2	6.5	5.3
R3	5.9	5.8
R4	5.7	5.9
R5	5.8	5.6
R6	5.7	5.4
R7	5.9	6.0
R8	6.2	6.2
R9	6.1	5.8
R10	5.8	5.4
Avg. length	**5.96±0.25**	**5.74±0.30**

Table 9: Length of silkworm larvae collected from Akalipur, Birbhum

Akalipur, Birbhum		
Sample No.	**Length of single larva, aghrani crop(cm)**	**Length of single larvae, falguni crop (cm)**
R1	6.0	6.2
R2	6.4	6.0
R3	6.2	6.4
R4	6.6	6.3
R5	6.5	6.2
R6	7.0	5.9
R7	6.0	5.8

R8	6.7	6.1
R9	6.5	6.2
R10	6.3	6.6
Avg. length	**6.42±0.31**	**6.17±0.23**

Percentage of length decrease in Aghrani crop:

i) C.S.R&T.I vs Akalipur = (6.42 – 5.71)/ (6.42) * 100%

= 11.05%

ii) Sahebnagar vs. Akalipur = (6.42 – 5.96)/ (6.42) * 100%

= 7.16%

Pertcentage of length decrease in Falguni crop:

i) C.S.R&T.I vs Akalipur = (6.17 – 5.64)/ (6.17) * 100%

= 8.58%

ii) Sahebnagar vs Akalipur = (6.17 – 5.74)/ (6.17) * 100%

= 6.96%

Chart no. 3: Average length of silkworm larvae among three locations (Aghrani crop)

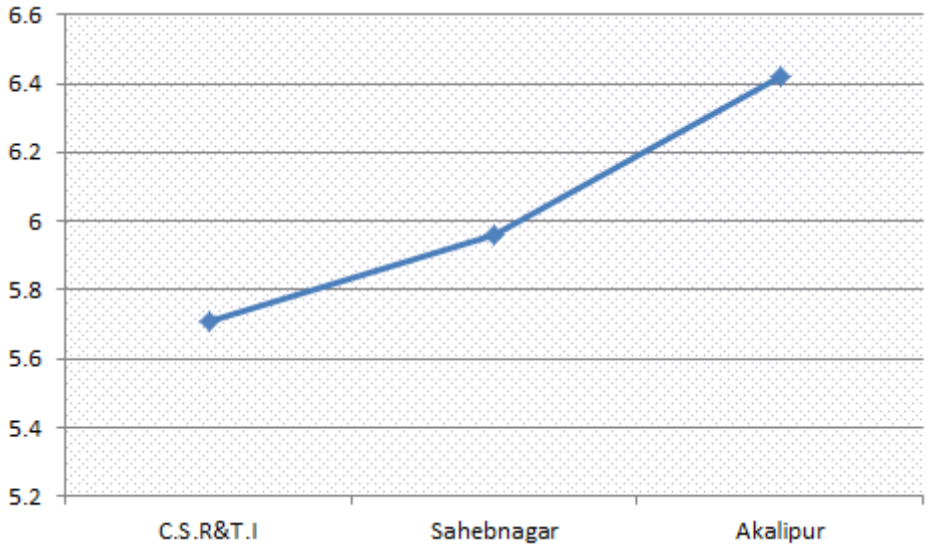
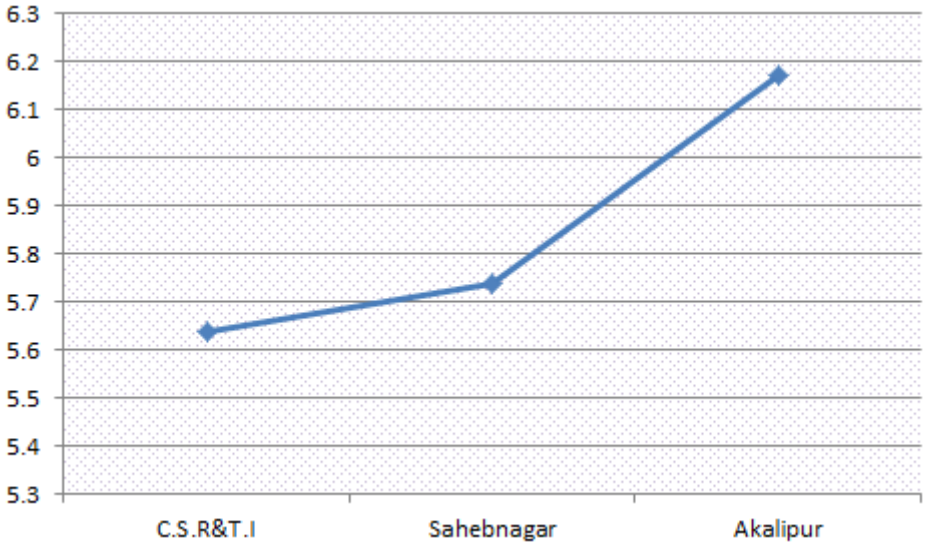


Chart no. 4: Average length of silkworm larvae among three locations (Falhuni Crop)



The data obtained from the above table and graph indicates that there has been a significant decrease in length of silkworm larvae in the arsenic contaminated areas (C.S.R&T.I and Sahebnagar). For both crop seasons Aghrani and Falguni, decrease in length has been identified in the arsenic contaminated zones. For the Aghrani crop, 11.05% and 7.16% decrease in length has been observed in silkworm larvae samples that were obtained from C.S.R&T.I Berhampore and Sahebnagar respectively. Whereas for the falguni crop, 8.58% and 6.96% decrease in length has been observed for the C.S. R&T.I and Sahebnagar samples. The findings of the study could be supported by the findings of Gintenreiter *et al*. (1993). In their study, they concluded that the growth, development and duration of the lifecycle of the larvae. In the experiment the larvae was fed with normal and nitrate fortified soil. The larvae fed with fortified leaves indicated slower food intake and simultaneous slow development. Similarly, Ashfaq *et al.* (2009b) and Shoukat *et al*. (2014) also reported a decrease in food consumption among silkworm larvae with increasing metal concentration on the leaves. Si *et al*. (2021) studied the accumulation of cadmium and lead in silkworm larvae, and identified a 26.8% decrease in length.

5.2 Estimation of total arsenic

5.2.1 Estimation of total arsenic concentration in collected water samples

The water sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected water samples are as follows:

Table 10: Estimation of total arsenic concentration in collected water samples

Water Sample no.	**C.S.R&T.I Berhampore, (mg/L)**	**Sahebnagar (Murshidabad) (mg/L)**	**Akalipur (Birbhum) (mg/L)**

R1	0.092±0.009	0.149±0.010	0.020±0.002
R2	0.095±0.009	0.172±0.010	0.019±0.002
R3	0.093±0.009	0.152±0.010	0.020±0.002
R4	0.110±0.009	0.140±0.010	0.019±0.002
R5	0.110±0.009	0.157±0.010	0.017±0.002
R6	0.110±0.009	0.150±0.010	0.025±0.002
Average conc.	**0.101±0.009**	**0.153±0.010**	**0.02±0.002**

From the above table, it could be identified that the arsenic levels in the water samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.101±0.009 mg/L, 0.153±0.010 mg/L, 0.02±0.002 mg/L respectively. The findings align with the results obtained by Adhikary and Mandal (2017) where they stated that the reported arsenic contamination in Murshidabad ranges between 0.05 mg/L to 0.9 mg/L. The permi9ssible limit for arsenic in consumable water however is limited to only 0.05 mg/L. Thus, while the obtained data indicates that the arsenic levels in the test areas are on the lower ranges, they surpass the permissible limit and could pose serious health hazards if consumed directly or indirectly for a prolonged period of time.

5.2.2 Estimation of total arsenic concentration in soil samples

The water sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected soil samples are as follows:

Table 11: Estimation of total arsenic concentration in soil samples

Sample no.	**C.S.R&T.I Berhampore (mg/kg)**	**Sahebnagar (Murshidabad) (mg/kg)**	**Akalipur (Birbhum) (mg/kg)**
R1	9.47±0.14	10.62±0.15	1.32±0.17

R2	9.62±0.14	10.57±0.15	1.50±0.17
R3	9.35±0.14	10.68±0.15	1.45±0.17
R4	9.32±0.14	10.82±0.15	1.38±0.17
R5	9.33±0.14	10.85±0.15	1.04±0.17
R6	9.65±0.14	10.97±0.15	1.51±0.17
Average conc.	**9.45±0.14**	**10.75±0.15**	**1.36±0.17**

From the above table, it could be identified that the arsenic levels in the soil samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 9.45±0.14 mg/kg, 10.75±0.15 mg/kg, 1.36±0.17 mg/kg respectively. Rahaman *et al*. (2013) conducted a study to understand arsenic contamination in West Bengal and understand its potential hazards. The authors noted that the total arsenic concentration in West Bengal to be around 13.12 mg/kg. The authors stated that while this exceeded the world average of 10.00 mg/kg, but is considered to be within acceptable limits as the acceptable limit for total arsenic in agricultural soils has been determined to be 20.00 mg/kg by the European Union.

5.2.3. Estimation of total arsenic concentration in leaf samples

The leaf sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected leaf samples are as follows:

Table 12: Estimation of total arsenic concentration in leaf samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.47±0.04	0.47±0.04	0.02±0.04
R2	0.40±0.04	0.45±0.04	0.04±0.04
R3	0.45±0.04	0.53±0.04	0.04±0.04

R4	0.48±0.04	0.48±0.04	0.02±0.04
R5	0.40±0.04	0.50±0.04	0.02±0.04
R6	0.48±0.04	0.50±0.04	0.03±0.04
Average Conc.	**0.42±0.04**	**0.50±0.04**	**0.02±0.04**

From the above table, it could be identified that the arsenic levels in the leaf samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.42±0.04 mg/kg, 0.50±0.04 mg/kg, 0.12±0.04 mg/kg respectively. Rahaman *et al*. (2013) indicated in their study that arsenic concentration in rice and potato was found to be around 0.42 mg/kg to 0.45 mg/kg.

5.2.4. Estimation of total arsenic concentration in larvae samples

The larvae sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected larvae samples are as follows:

Table 13: Estimation of total arsenic concentration in larvae samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	1.75±0.30	1.68±0.11	0.32±0.04
R2	1.70±0.30	1.82±0.11	0.34±0.04
R3	1.67±0.30	1.85±0.11	0.29±0.04
R4	1.78±0.30	1.94±0.11	0.37±0.04
R5	1.17±0.30	1.97±0.11	0.39±0.04
R6	1.11±0.30	1.96±0.11	0.39±0.04
Average	**1.53±0.30**	**1.87±0.11**	**0.35±0.04**

Conc.			

From the above table, it could be identified that the arsenic levels in the larvae samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 1.53±0.30 mg/kg, 1.87±0.11 mg/kg, 0.35±0.04 mg/kg respectively. While accumulation of arsenic in silkworm has not been extensively studied extensively, there has been an extensive study regarding the accumulation of chromium and cadmium in silkworm larvae. Si *et al*. (2021) conducted a research to understand the accumulation of lead and cadmium in the mulberry-silkworm food chain. They found out that 94.98 mg/kg and 1.62 mg/kg of lead and cadmium was accumulated in silkworm larvae. Ashfaq *et al*. (2012) also reported 61.32 mg/kg chromium was deposited in silkworm larvae. The study indicated that the accumulation of heavy metal was higher in the larvae as compared to the leaves.

5.2.5. Estimation of total arsenic concentration in excreta samples

The excreta sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected excreta samples are as follows:

Table 14: Estimation of total arsenic concentration in excreta samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.70±0.05	0.71±0.02	0.23±0.04
R2	0.75±0.05	0.75±0.02	0.25±0.04
R3	0.65±0.05	0.68±0.02	0.19±0.04
R4	0.68±0.05	0.72±0.02	0.15±0.04
R5	0.75±0.05	0.75±0.02	0.25±0.04
R6	0.79±0.05	0.75±0.02	0.25±0.04
Average Conc.	**0.72±0.05**	**0.73±0.02**	**0.23±0.04**

From the above table, it could be identified that the arsenic levels in the excreta samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.72±0.05 mg/kg, 0.73±0.02 mg/kg, 0.23±0.04 mg/kg respectively. While extensive study has not been undertaken to understand the accumulation of arsenic, in silkworm excreta, other heavy metals like chromium has been studied. Ashfaq *et al*. (2012) have indicated in their study that about 58.95 mg/kg bio-accumulated in silkworm faeces.

5.2.6. Estimation of total arsenic concentration in silk samples

The silk sample for arsenic contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the test area for total arsenic analysis, Akalipur was considered as the control area. The data obtained from the analysis of the collected silk samples are as follows:

Table 15: Estimation of total arsenic concentration in silk samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	3.67±0.25	5.07±0.34	0.42±0.04
R2	3.89±0.25	5.65±0.34	0.50±0.04
R3	4.02±0.25	5.87±0.34	0.52±0.04
R4	4.08±0.25	6.02±0.34	0.55±0.04
R5	4.37±0.25	5.72±0.34	0.56±0.04
R6	4.27±0.25	5.39±0.34	0.51±0.04
Average Conc.	**4.05±0.25**	**5.62±0.34**	**0.51±0.04**

From the above table, it could be identified that the arsenic levels in the excreta samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 4.05±0.25 mg/kg, 5.62±0.34 mg/kg, 0.51±0.04 mg/kg respectively. A study condicted by Baidya *et al.* (2019) indicates the accumulation of 20.80 mg/kg chromium in the silk thread. The study indicates a similar patter where the maximum amount of heavy metal gets deposited in the silk following the mulberry-silkworm food chain.

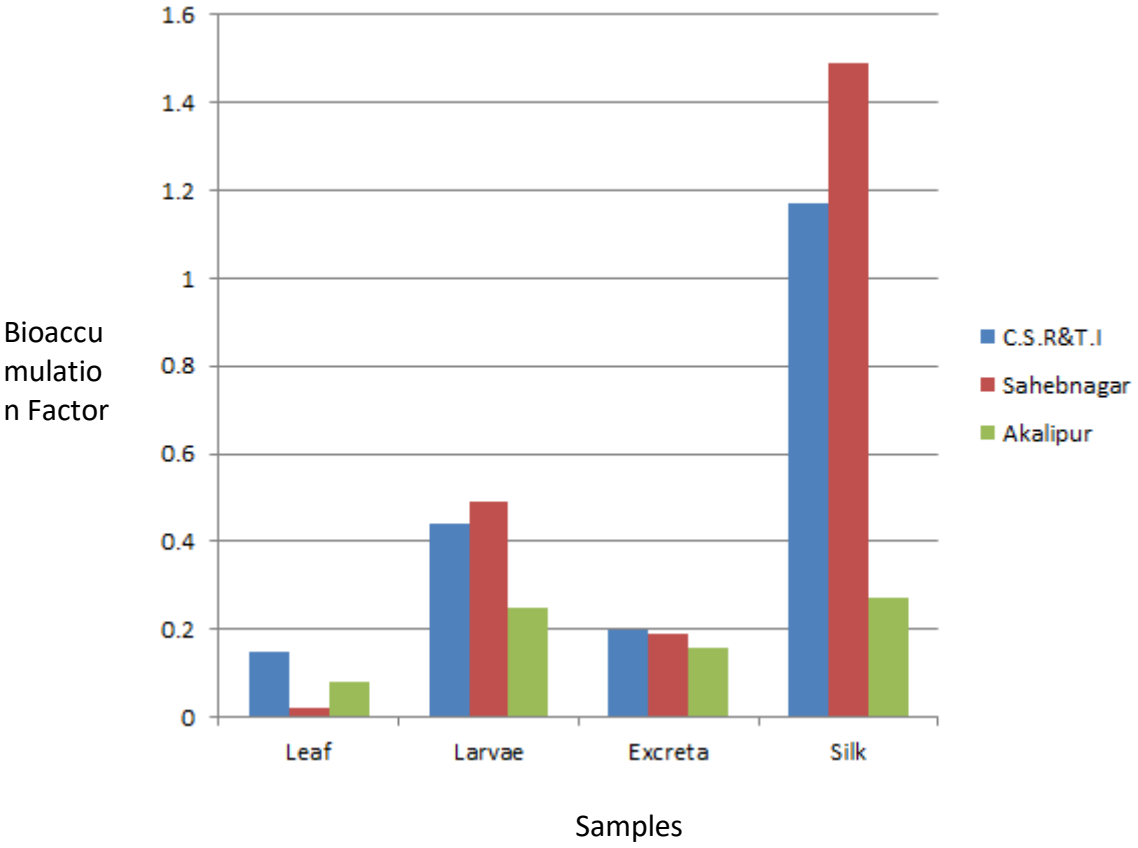
5.2.7. Bioaccumulation factor for total arsenic

The bioaccumulation factor for total arsenic has been studied. The data obtained are as follows:

Table 16: Estimation of bioaccumulation factor of total arsenic

Samples	C.S.R&T.I Berhampore (As contaminated)	Sahebnagar, Murshidabad (As contaminated)	Akalipur, Birbhum (control)	Bioaccumulation factor (C.S.R&T.I)	Bioaccumulation factor (Sahebnagar)	Bioaccumulation factor (Akalipur)
Water	0.101±0.009	0.153±0.010	0.02±0.002	-	-	-
Soil	9.45±0.14	10.75±0.15	1.36±0.17	-	-	-
Leaf	0.42±0.04	0.50±0.04	0.02±0.04	0.04	0.04	0.01
Larvae	1.53±0.30	1.87±0.11	0.35±0.04	0.44	0.49	0.25
Excreta	0.72±0.05	0.73±0.02	0.23±0.04	0.20	0.19	0.16
Silk	4.05±0.25	5.62±0.34	0.51±0.04	1.17	1.49	0.37

Chart no.5: Graphical representation of the bioaccumulation factor for total arsenic



From the above table, it could be concluded that the bioaccumulation factor is highest in the samples that have been obtained from Sahebnagar (test area), followed by C.S.R&T.I (test area) and Akalipur (control area). The findings could be confirmed by the findings of Perugini *et al.* (2011) where the study indicated a higher accumulation of heavy metals in the food chain of honey bee that were collected from Ciampino which is an urban area.

5.3. Estimation of Fluoride

5.3.1. Estimation of fluoride concentration in collected water samples

The water sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected water samples are as follows:

Table 17: Estimation of fluoride contamination in water samples

Sample no.	C.S.R&T.I Berhampore (mg/L)	Sahebnagar, Murshidabad (mg/L)	Akalipur, Birbhum (mg/L)
R1	0.04±0.01	0.06±0.01	1.24±0.11
R2	0.03±0.01	0.06±0.01	1.68±0.11

R3	0.04±0.01	0.06±0.01	1.57±0.11
R4	0.05±0.01	0.06±0.01	1.58±0.11
R5	0.07±0.01	0.07±0.01	1.62±0.11
R6	0.07±0.01	0.7±0.01	1.41±0.11
Average Conc.	**0.05±0.01**	**0.06±0.01**	**1.60±0.11**

From the above table, it could be identified that the fluoride levels in the water samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.05±0.01 mg/L, 0.06±0.01 mg/L, 1.60±0.11 mg/L respectively. The finding could be supported by the study conducted by Batabyal and Gupta (2017). The authors have stated that the concentration of fluoride contamination in Birbhum ranges from 0.01 to 18 mg/L in the pre-monsoon period and from 0.023 to 19 mg/L in the post monsoon period. Mukherjee *et al.* (2016) have indicated that fluoride contamination in Birbhum (in groundwater) ranges from 2.88 mg/L to 9.36 mg/L. The data obtained for Birbhum water sample indicates fluoride contamination on the lower ranges could be dedicated to the fact that the water was collected from a surface water source (Bramhani River) as compared to groundwater like the mentioned researches. On the contrary, the fluoride contamination is negligible in the water samples taken from Murshidabad as they are control areas with no proven of fluoride contamination in the district.

5.3.2. Estimation of fluoride concentration in collected soil samples

The soil sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected soil samples are as follows:

Table 18: Estimation of fluoride concentration in soil samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.07±4.33	0.08±0.00	192±3.03
R2	0.04±4.33	0.07±0.00	187±3.03

R3	0.03±4.33	0.09±0.00	190±3.03
R4	0.06±4.33	0.07±0.00	195±3.03
R5	0.05±4.33	0.09±0.00	188±3.03
R6	0.02±4.33	0.09±0.00	188±3.03
Average Conc.	**0.04±0.01**	**0.08±0.00**	**190±3.03**

From the above table, it could be identified that the fluoride levels in the soil samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.04±0.01 mg/kg, 0.08±0.00 mg/kg, 190±3.03 mg/kg respectively. Results obtained by Bhattacharya and Samal (2018) indicates that fluoride concentration in fluoride contamination in soil ranges from 20-500 mg/kg while 1000 mg/kg could be hired from soils that are obtained from rocks with higher fluorine content or from soils that are irrigated with phosphate fertilizers. According to Bhat *et al*. the fluoride contamination could arise from phosphorous fertilizers which contains 1% to 1.5% fluorine.

5.3.3. Estimation of fluoride concentration in collected leaf samples

The leaf sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected leaf samples are as follows:

Table 19: Estimation of fluoride concentration in leaf samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.07±0.02	0.07±0.01	3.75±0.10
R2	0.02±0.02	0.07±0.01	3.80±0.10
R3	0.01±0.02	0.05±0.01	3.97±0.10
R4	0.03±0.02	0.05±0.01	3.99±0.10

R5	0.03±0.02	0.05±0.01	3.78±0.10
R6	0.02±0.02	0.07±0.01	3.89±0.10
Average Conc.	**0.03±0.02**	**0.06±0.01**	**3.86±0.10**

From the above table, it could be identified that the fluoride levels in the leaf samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.03±0.02 mg/kg, 0.06±0.01 mg/kg, 3.86±0.10 mg/kg respectively. The study carried out by Pal *et al*. indicated the accumulation of fluoride in leafy vegetables that are grown on polluted soil to be 4.12 mg/kg. The decrease in value in the current study could be justified by the fact that farmers mostly use surface water(Bramhani River) for irrigation which has a significantly low fluoride concentration as compared to groundwater.

5.3.4. Estimation of fluoride concentration in collected larvae samples

The larvae sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected larvae samples are as follows:

Table 20: Estimation of fluoride concentration in larvae samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.01±0.00	0.05±0.02	5.04±0.02
R2	0.01±0.00	0.07±0.02	5.00±0.02
R3	0.02±0.00	0.05±0.02	5.02±0.02
R4	0.02±0.00	0.02±0.02	5.02±0.02
R5	0.01±0.00	0.08±0.02	5.06±0.02
R6	0.01±0.00	0.05±0.02	5.01±5.02

Average Conc.	**0.01±0.00**	**0.05±0.02**	**5.02±0.02**

From the above table, it could be identified that the fluoride levels in the larvae samples collected from C.S.R&T.I, Sahebnagar and Akalipur are 0.01±0.00 mg/kg, 0.05±0.02 mg/kg, 5.02±0.02 mg/kg respectively. While accumulation of arsenic in silkworm has not been extensively studied extensively, there has been an extensive study regarding the accumulation of chromium and cadmium in silkworm larvae. Si *et al*. (2021) conducted a research to understand the accumulation of lead and cadmium in the mulberry-silkworm food chain. They found out that 94.98 mg/kg and 1.62 mg/kg of lead and cadmium was accumulated in silkworm larvae. Ashfaq *et al*. (2012) also reported 61.32 mg/kg chromium was deposited in silkworm larvae. The study indicated that the accumulation of heavy metal was higher in the larvae as compared to the leaves.

5.3.5. Estimation of fluoride concentration in collected excreta samples

The excreta sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected excreta samples are as follows:

Table 21: Estimation of fluoride concentration in collected excreta samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.001±0.00	0.00±0.00	2.01±0.01
R2	0.001±0.00	0.0001±0.00	2.02±0.01
R3	0.001±0.00	0.0001±0.00	2.01±0.01
R4	0.001±0.00	0.0001±0.00	2.05±0.01
R5	0.001±0.00	0.0001±0.00	2.04±0.01
R6	0.001±0.00	0.0001±0.00	2.01±0.01
Average Conc.	**0.001±0.00**	**0.001±0.00**	**2.02±0.01**

From the above table, it could be identified that the fluoride levels in the larvae samples collected from C.S.R&T.I, Sahebnagar and Akalipur are approximately 0.001±0 mg/kg and 2.02±0.01 mg/kg respectively. While extensive study has not been undertaken to understand the accumulation of arsenic, in silkworm excreta, other heavy metals like chromium has been studied. Ashfaq *et al*. (2012) have indicated in their study that about 58.95 mg/kg bio-accumulated in silkworm faeces.

5.3.6. Estimation of fluoride concentration in collected silk samples

The excreta sample for fluoride contamination was collected from three locations. While C.S.R&T.I and Sahebnagar served as the control area for fluoride analysis, Akalipur was considered as the test area. The data obtained from the analysis of the collected excreta samples are as follows:

Table 22: Estimation of fluoride concentration in silk samples

Sample no.	C.S.R&T.I Berhampore (mg/kg)	Sahebnagar, Murshidabad (mg/kg)	Akalipur, Birbhum (mg/kg)
R1	0.001±0.00	0.001±0.00	4.07±0.02
R2	0.001±0.00	0.001±0.00	5.04±0.02
R3	0.001±0.00	0.001±0.00	5.09±0.52
R4	0.001±0.00	0.001±0.00	4.03±0.52
R5	0.001±0.00	0.001±0.00	4.03±0.52
R6	0.001±0.00	0.001±0.00	4.03±0.52
Average Conc.	**0.001±0.00**	**0.001±0.00**	**4.38±0.52**

From the above table, it could be identified that the fluoride levels in the larvae samples collected from C.S.R&T.I, Sahebnagar and Akalipur are approximately 0.001±0 mg/kg, 4.38±0.52 mg/kg respectively. A study conducted by Baidya *et al.* (2019) indicates the accumulation of 20.80 mg/kg chromium in the silk thread. The study indicates a similar patter where the maximum amount of heavy metal gets deposited in the silk following the mulberry-silkworm food chain.

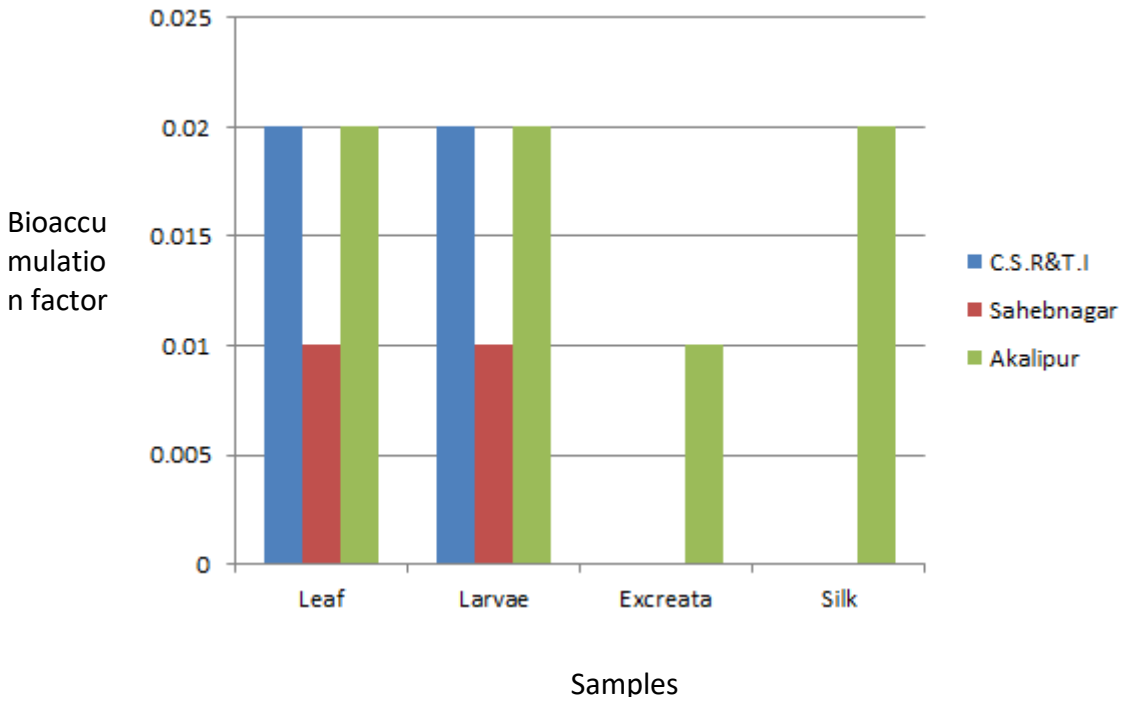
5.3.7. Bioaccumulation factor for fluoride

The bioaccumulation factor for total arsenic has been studied. The data obtained are as follows:

Table 23: Bioaccumulation factor for fluoride

Samples	C.S.R&T.I Berhampore (Control area)	Sahebnagar, Murshidabad (Control area)	Akalipur, Birbhum (Fluoride contaminated)	Bioaccumulation factor (C.S.R&T.I)	Bioaccumulation factor (Sahebnagar)	Bioaccumulation factor (Akalipur)
Water	0.05±0.01	0.06±0.01	1.6±0.11	-	-	-
Soil	0.04±0.01	0.08±0.00	190±3.03	-	-	-
Leaf	0.001±0.02	0.001±0.01	3.86±0.10	0.02	0.01	0.02
Larvae	0.001±0.00	0.001±0.02	5.02±0.02	0.02	0.01	0.02
Excreta	<0.001	<0.001	2.02±0.01	<0.01	<0.01	0.01
Silk	<0.001	<0.001	4.38±0.52	<0.01	<0.01	0.02

Chart no. 6: Graphical representation of the bioaccumulation factor for fluoride



From the above table, it could be concluded that the bioaccumulation factor is highest in the samples that have been obtained from Akalipur (test area), followed by C.S.R&T.I (control area) and Sahebnagar (control area). The findings could be confirmed by the findings of Perugini *et al.* (2011) where the study indicated a higher accumulation of heavy metals in the food chain of honey bee that were collected from Ciampino which is an urban area.

Chapter

6. Conclusion

From the above study, it could be concluded that the mulberry-silkworm food chain could serve as an effective tool to remediate soils that are contaminated with arsenic and fluoride. From the findings of the study, it could be concluded that the physical characteristics of the silkworm larvae has been impacted significantly. The significant decrease in weight and length of the silkworm larvae has however been identified in the arsenic affected areas (C.S.R&T.I and Sahebnagar) as compared to the fluoride-contaminated zone (Akalipur, Birbhum). This finding could be supported by the evidences that could be found in previous literature where researchers. They have identified that consumption of leaves that are fortified with heavy metals impact the larvae's food consumption levels; as a result, their overall development is affected. The concentration of total arsenic in the soil was found to be 9.45±0.14 mg/kg and 10.75±0.15 mg/kg in the test areas (C.S.R&T.I Berhampore and Sahebnagar respectively). The data obtained from this study identified that the mulberry-silkworm food chain has been successful in remediating a significant amount of total arsenic amounting to 4.05 mg/kg and 5.62 mg/kg (C.S.R.&T.I Berhampore and Sahebnagar respectively) and has passed it on to a completely different food chain. Same phenomenon could be identified in the fluoride-contaminated zone. The findings of this study indicated that 4.38±0.52 mg/kg fluoride was remediated from the contaminated soil (Akalipur) with the help of the mulberry-silkworm food chain. While the amount of fluoride remediated from the test area seems to be significant, it is extremely minute in terms of the heavy metal accumulation in soil (190±3.03 mg/kg). In the case of total arsenic, the BCF is greater than one (>1), which indicates that mulberry-silkworm food chain is suitable for arsenic phytoremediation. However, in the case of fluoride, it could be seen that the BCF value is lower than one (<1) which indicates that the mulberry-silkworm food chain could be used for phytostabilization of fluoride instead of phytoremediation. Thus, it could be concluded that the mulberry-silkworm food chain is much more effective for phytoremediation of arsenic contaminated soils as compared to fluoride-contaminated soils and could be used to prevent the entry of such heavy metals into the human food chain, which causes serious threats to human health.

Future Prospects

This study could be extended in the future by studying the effectiveness of different silkworm breeds for the phytoremediation process. While only one silkworm breed has been considered for this study, several other silkworm breeds are reared in India. Analysing and comparing the effectiveness of these breeds for the phytoremediation of soils that are polluted with heavy metals could help to identify the silkworm breeds that are most effective for the phytoremediation process.

Chapter

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