G.J.B.B., VOL.7 (3) 2018: 490-498 ISSN 2278 – 9103

GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

STUDIES ON ASSESSMENT OF HEAVY METALS IN SAMPLES COLLECTED FROM SURROUNDING AREA OF RECYCLING PLANT IN MADURAI DISTRICT OF SOUTHERN INDIA

S. Muralikrishnan[1] P. Madhan[1] N. Arun nagendran[1] D. Pandiaraja[1] T. Kubendran[2*]
[1]National Centre of Excellence on Statistical and Mathematical Modeling on Bioresources Management-MHRD, Thiagarajar College, Madurai-625009, Tamil Nadu, India
[2]High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan-173 211, Himachal Pradesh, India.
*Corresponding author email: tkbaetis@gmail.com

ABSTRACT
From waste to energy generation is considered as potential approach to resolve the challenges associated with waste Food safety is considered as a major concern, public as well as governmental agencies in the developed countries are very much fearful about accumulation of heavy metals in food chain. In the present study the temperature of the tap water recorded $27^0C \pm 2^0C$ and the temperature of the sewage water was observed at $27.5^0C \pm 2^0C$. The pH level of tap water and sewage water is 6.68 & 6.62 respectively. The relative abundance of total heavy metals in waste water, tap water and five plant species were found in the order Cu, Cd & Zn. In addition to that from water and soil samples six different bacterial colonies were isolated and few biochemical characterizations were analyzed.

KEY WORDS: Heavy metals, microbial analysis, recycling plant, Plant species.

INTRODUCTION

Water scarcity is one of the most demanding problem of today. It has been estimated that one in three persons will face water scarcity by the year 2025 in India (IWMI 2003) or around 2.7 billion people worldwide by the same time (UN Report 2003). The pollution of heavy metals causes potential ecological risk in different ecosystems. The base of phytoremediation, a promising method for cleaning of soil and water, is pollutant uptake or bounding by plants. Initially much more interest was focused on hyper-accumulator plants than those taken in non-accumulator plants **(**Baker *et al.,* 1994; Brooks 1998)**.** Later, as an alternative to substitution of the endemic, hardly cultivable plants accumulator plants have been used; possibly coupled with treatment of soil, either considering factors to increase the metal availability and plant uptake (Deram *et al.,* 2000; Nemeth *et al.,* 1993). Other possibility to decrease available concentration of pollutants is stabilization. Phytostabilisation can gain results from either physical or chemical effects of plants and of chemicals, such beignet, lime or clay minerals (Gworek 1997; Lehoczky *et al.,* 2000). The tested plant species were grouped on the basis of their accumulation capability and susceptibility of heavy metals. Results assist to elaborate a possible combination of Phytoextraction and Phytostabilisation technology under the present ecological conditions. There are 35 metals that are of concern for us because of residential or occupational exposure, out of which 23 are heavy metals: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc (Mosby *et al.,* 1996). These heavy metals are commonly found in the environment and diet. In small amounts they are required for maintaining good health but in larger amounts they can become toxic or dangerous. Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs. Long-term exposure can lead to gradually progressing physical, muscular and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy. Repeated long-term exposure of some metals and their compounds may even cause cancer (Jarupl, 2003). The toxicity level of a few heavy metals can be just above the background concentrations that are being present naturally in the environment. Hence thorough knowledge of heavy metals is rather important for allowing providing proper defensive measures against their excessive contact (Ferner, 2001). The rapid development of urbanization and industrialization, together with the shortage of availability of fresh water for irrigation led to the rising use of sewage for agricultural land irrigation. Reuse of domestic and industrial wastewater in agriculture for irrigating crops appears to be a lucrative or profitable option. Besides being a source of irrigation water, this wastewater contains appreciable amounts of plant nutrients. This wastewater may also carry appreciable amounts of trace toxic metals and its long term application on agricultural lands contributes significantly to the buildup of elevated concentration of toxic metals in irrigated soil and plants (Rattan, 2005). Crop species exercise differentially in accumulating metals in their tissue and efficiency of different crops in absorption of metals is judged either by plant metal uptake or by transfer factor of metals from soil to plants. The process of plant growth depends on the cycle of nutrients including trace elements from soil to plant. Heavy metal accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metals and is evaluated by either plant uptake or soil-to-plant transfer factors of the metals (Rattan, 2005). The metal accumulation in different plant parts depends on the availability and chemical form of metals in soil, their translocation potential, and type of plant species with their stage of maturity. A number of factors such as climate, atmospheric deposition, the concentrations of heavy metals in soil, the nature of soil on which vegetables are grown also affects bio-concentration of heavy metals in vegetables. Human diet as they contain essential components needed by the human body such as carbohydrates, proteins, vitamins, minerals and also trace elements. Excessive accumulation of metals in vegetables may pose serious threat to the local residents, who consume crops or vegetables grown in contaminated area (Chary *et al.,* 2008). Consumption of vegetables is one of the pathways by which heavy metals enter the food chain (Wang, 2005). Excessive accumulation of dietary heavy metals such as cadmium, lead and chromium can lead to serious health problems. Heavy metals persist in the environment, are non-biodegradable and have the potential to accumulate in different body organs. The consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intrauterine growth retardation, impaired.

Psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates (Khan *et al.,* 2013). In India, there are more than 200 sewage farms covering an area about 50,000 hectares. The present study focuses on the heavy metal uptake by plants in sewage irrigated peri-urban agricultural land in Vellakal region, Avanyapuram, Madurai. In this region sewage irrigation has been practiced since 1924. Various leafy vegetables and fodder crops have been successfully grown and are being consumed. Plants are important components of ecosystems as they transfer elements from abiotic into biotic environments. The primary sources of elements from the environment to plants are: air, water and the soil (Hamilton, 1995). Among the elements, the most important to consider in terms of food chain contamination are As, Cd, Hg and Pb. Simultaneously, some micronutrient elements (e.g. Cu, Cr, Ni, Zn) may be toxic to both plants and animals at high concentration. The bioavailability of elements to plants is controlled by many factors associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer processes. Each heavy metal shows specific signs of its toxicity. For instance Pb, As, Hg, Zn, Cu, Cd and Al poisoning have been implicated with gastrointestinal disorders, diarrhea, vomiting and convulsion, depression and pneumonia. Some effects of heavy metals could be toxic or even carcinogenic. Soils as filters of toxic chemicals may adsorb and retain heavy metals from wastewater. However, when the capacity of soil is reduced to retain the toxic metals due to the continuous loading of pollutants or changes in pH, soils can release heavy metals in to ground water or soil solution available for plant uptake. The present study area, metal accumulation in different plants species cultivated in Avanyapuram sewage farm near to the Madurai waste dumping area. In this area many plant (spinach) species and grasses for domestic animals (cow, goat) are cultivated by using sewage wastewater. Besides being a source of irrigation water, this wastewater contains appreciable amounts of plants nutrients. This wastewater may also contain appreciable amount of trace toxic metals. The metal accumulation in different plant parts depends on the availability and chemical from the soil, their translocation potential, and type of plant species with stage of maturity. Based upon the above consequences, an attempt was made in order to analyze the heavy metals present in water, soil and plant species located at Avanyapuram recycling plant area, Madurai.

MATERIALS & METHODS

Study area

Madurai is one of the oldest continuously inhabited cities in the Indian peninsula. Madurai city has an area of 52 km^2, within an urban area now extending over as much as 130 km^2 and it is located at 9°49'43" to 9° 54'25" N and 78°04'32" to 78°08'09" E. It is an ancient and prestigious city in the Indian state of Tamil Nadu and situated on the banks of the river Vaigai**.**

Water quality parameters

Physico-chemical parameters were analyzed by following standard protocols described by Trivedy and Goel (1986). Temperature was recorded by using thermometer for water samples. pH was noticed for both sewage water and tap water by using the digital pH meter. Dissolved oxygen was noted down by Winkler's iodometric method. To observe the presence of atoms/molecules in the samples, both sewage and tap water collected from the study area was subjected to UV-Spectrophotometer analysis at 540 nm.

Collection of samples

Rhizosphere soil, sewage water (used in agriculture) tap water and five spinach species were collected from agricultural area in Vellakal, Avanyapuram, Madurai (Plate 1). The tasting was carried out in the month of mid-October and end of November (post monsoon season). The polyethylene bags were used for the accumulation of plant stuff (leaves & petiole) and soil samples, whereas the sewage water and tap water were collected in plastic bottles. Total ten numbers of samples were collected in and round the recycling plant for the assessment of bacteria, fungi and heavy metals. The experiment was carried out for soil samples, sewage water and tap water and 5 plant species.

Analysis heavy metals:

To examine the bioavailability of heavy metals, the plant species viz. *Amaranths tricolor* Linn. *Amaranths tender* Linn. *Tropical amaranth* Linn. *Alternanthera sessilis* Linn. *Spinacia oleracea* Linn. were collected from the surrounding areas of recycling plant. The leaves and petiole were separate and grid with the help of mortar and pestle after drying. All the samples were subjected to the heavy metal analysis in the laboratory. The plant materials were washed with tap water thoroughly and sun dried for 2 to 4 days and soil samples were oven dried at 80℃ at overnight. The dried samples were ground with mortar and pestle. The digestion was carried out for water and plant samples by using HNO_3, H_2SO_4 and $HClO_3$ in the ratio 5:1:1 respectively at 80^0 C until a transparent solution was obtained. The prepared water and plant sample are used for analysis of Cu, Zn and Cd were carried away by using (AAS) Atomic Absorption Spectrophotometry (Model: AA-7000 SHIMADZU) at National Centre of Excellence, Thiagarajar College, Madurai.

Microbial analysis

The isolation of bacteria and fungi was achieved by serial dilution and spread plate techniques. Serial dilution was done for all samples (soil sample, tap water, sewage water). For bacterial isolation nutrient agar medium was used whereas for fungi isolation potato dextrose agar medium was used. The bacterial isolates were subjected to Gram's staining procedure and various biochemical tests *viz*., indole test, methyl red test, Voges- Proskauer test, Simmons citrate test based on the standard protocols described by Cappuccino and Sherman (2005) were analysed. The fungal colonies grown on all the petri dishes were counted and identified. The colonies were stained by Lacto phenol cotton blue solution and microscopic morphology was identified. The mycelial and conidial structures was observed under microscope and used for identification.

RESULTS & DISCUSSION

Physico-chemical parameters play an important role in determining the distributional pattern and quantitative abundance of organisms inhabiting a particular ecosystem. The following Physico-chemical parameters were analyzed in the present study. Temperature plays a vital role in the growth of all the living organisms. The temperature of the tap water recorded 27^0C ± 2^0C and the temperature of the sewage water was observed at 27.5^0 C ± 2^0C. Optimal water temperature for growth is 28-30^0C (Center *et al.*, 2002). Temperatures above 33^0C inhibit further growth (Center *et al.*, 2002). Optimal air temperature is 21-30^0C (U.S. EPA, 1988). If lasting for 12 hours temperature of -3^0C will destroy all leaves and temperature of -5^0C during the period of 48hours will destroy whole plant (Jaikumar, 2012; U.S. EPA, 1988). Determination of pH plays an important role in the waste water treatment process. Extreme levels, presence of particular matters, accumulations of toxic heavy metals and increase in alkalinity levels may cause change in the neutral pH value. Hence, in the particular analysis pH level of tap water and sewage water was found to be 6.68, 6.62 respectively. pH was observed to be acidic in both tap and sewage water because decomposition of organic matter lowers the pH. Optimal water pH for growth of this plant isneutral but it can tolerate pH values from 4 to 10 that *Eichhornia crassipes* can be used for treatment of different types of wastewater. (Jaikumar 2012; Center, *et al.*, 2002).

PLATE 1. Location and collection of samples at waste water recycling plant area located at Vellakal, Avanyapuram, Madurai

This is very important fact because it points in India, due to sulfur rich coal the pH was observed alkaline and the high salt content can increase the electrical conductivity (μs/cm) (Nalawade *et al.,* 2015). The pathogens of microbes are also significantly reduced through the highly toxic potential and near neutral pH together with the ecology of the environment (Golda *et al,* 2014; Kadam *et al.,* 2008; 2009). The turbidity of tap water and sewage water was examined by UV-spectrophotometer. Tap water revealed transmittance range of 0.02 at 540 nm whereas sewage water samples with greenish brown showed transmittance range of 0.06 at 540 nm. The employment of optical electronic systems for the monitoring of waste water treatment process is a recent approach. Dissolved oxygen is an important parameter in assessing water quality because of its influence on the organisms living within the body of water. The dissolved oxygen for tap and sewage water was recorded as 4 and 0.1 mg/L. In order to metabolize food and reproduce, each microorganism must have at least 0.1 to 0.3 mg/L of dissolved oxygen. The dissolved oxygen was found to be very low in sewage water due to the deposition of suspended particles.The dissolvedoxygen concentration in the streams ranged from 8.07 to10.64 mg/ L and 8.8 to 9.79 mg/L. In Kurangani stream, maximum of 10.64 mg/L of dissolved oxygen was recorded (Kubendran and Ramesh, 2016a,b). Maximum dissolved oxygen was observed during post monsoon season but minimum temperature was recorded in Konthagai pond (Muralikrishnan *et al*., 2017). Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. The dissolved oxygen was lesser in some polluted water and high in undisturbed water ecosystem. Dissolved oxygen levels change and vary according to the time of day, the weather and the temperature (Balachandran and Ramachandra, 2011; Kubendran *et al.*, 2017a, b).

Bacterial colonies isolation

In the present study by using collected water and soil samples, six different bacterial colonies were isolated and few biochemical characterizations were done (Table 1; Plate 2 & 3). Based upon the Lacto phenol cotton blue staining and hyphae structure the fungal species were identified as *Aspergillus niger* and *Mycorrhizal* fungi was identified from sewage and soil sample respectively (Plate 4).

Mycorrhizal fungi colonize the plant's root system and develop a symbiotic association called "mycorrhizae" mycorrhizae form a network of filaments that associate with plant roots and draw nutrients and water from the soil that the root system would not be able to access otherwise. The Copper concentration in *Amaranthusaritis, Alternanthera sessilis, Spinacia oleracea, Amaranthus polygonoides, Amaranthus tricolor*, sewage water, tap water were 0.504, 0.602, 0.432, 0.288, 0.408, 0.264, 0.360 mg/L respectively (Figure 1.1).

TABLE 1: Biochemical Characterization for Isolated Microbial Colonies

Test	Water (T1)	Soil				
		Pink (T2)	Thick Yellow (T3)	Light Yellow (T4)	Thick White (T5)	Light White (T6)
Gram's Staining	N	P	P	N	P	P
Indole Test	P	P	N	N	P	P
Methyl Red Test	P	P	P	P	N	P
Voges Proskauer	N	P	P	P	P	P
Citrate Utilization	P	P	P	P	P	P

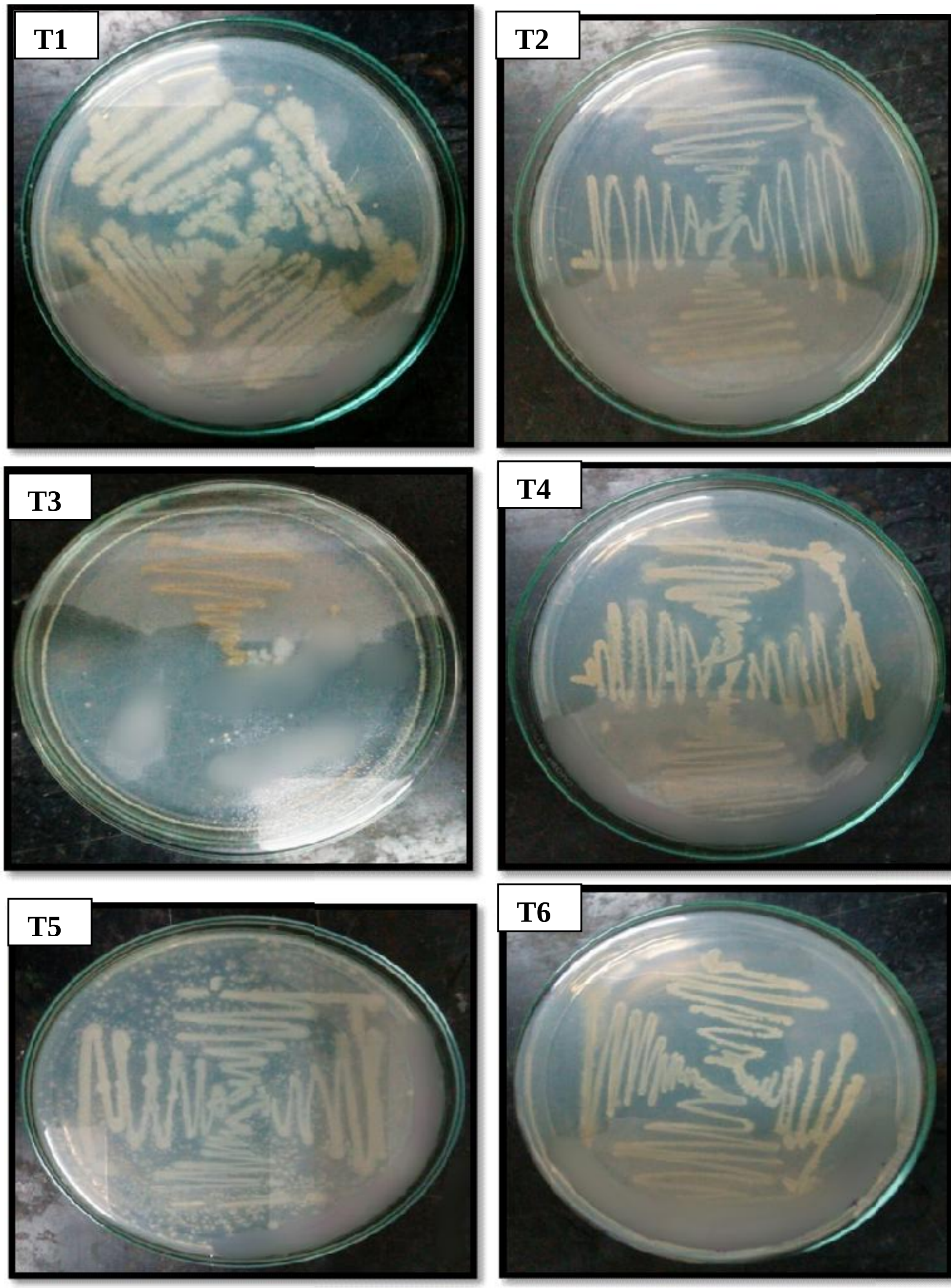
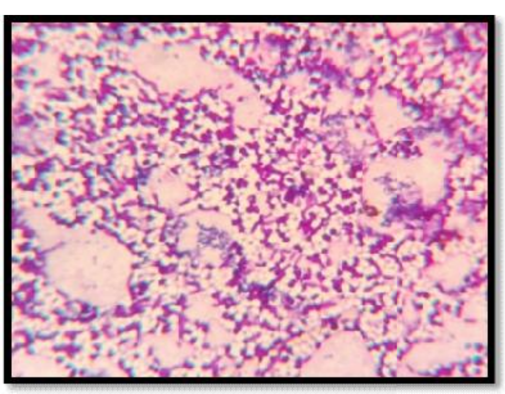
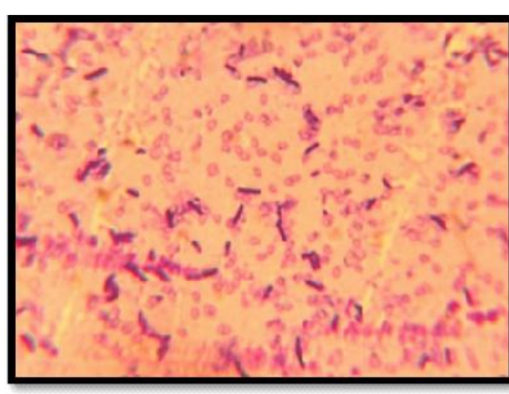


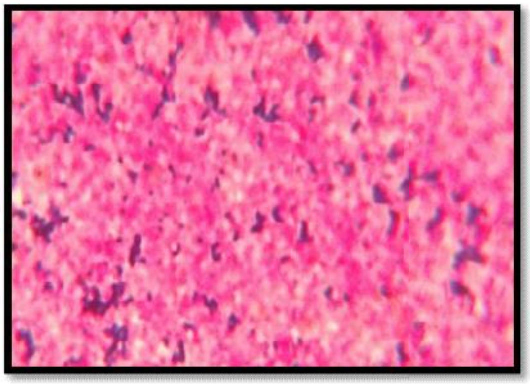
PLATE 2. Isolation of six different bacterial colonies from water and soil samples from waste water recycling plant area located at Vellakal, Avanyapuram, Madurai



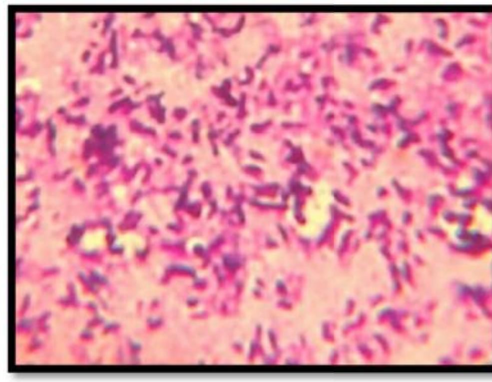
T1



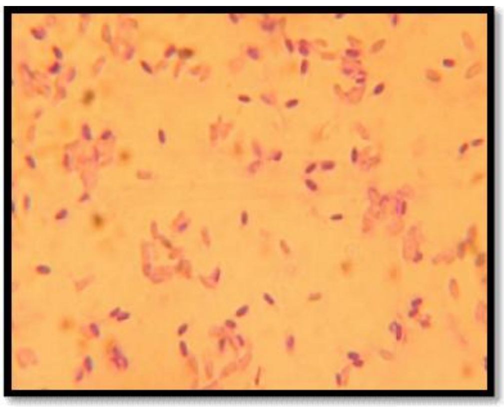
T2



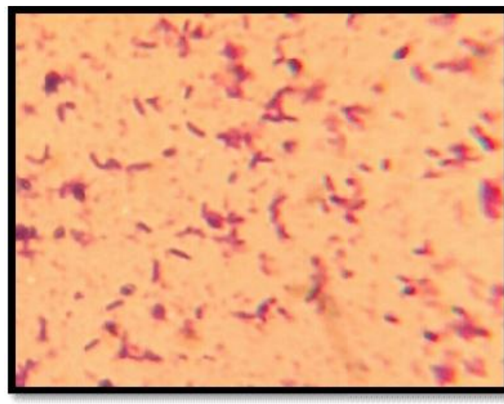
T3



T4

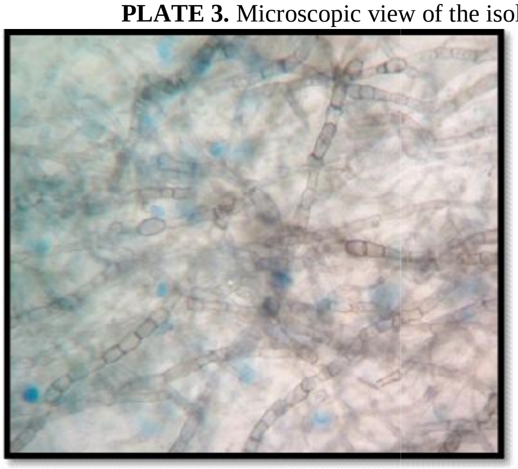


T5



T6

PLATE 3. Microscopic view of the isolated bacterial colonies by Gram's staining method



a

b

PLATE 4. Microscopic hyphae view of a) *Aspergillus niger* b) *Mycorrhiza*

Copper concentration of *Alternantherasessilis* (0.602 mg/L) reached beyond the permissible limit denoted by the BIS (Permissible limit-0.05 mg/L). The concentration of Copper was found to be highest in *Alternantherasessilis* (0.602 mg/L) which is followed by *Amaranthusaritis* (0.504 mg/L), *Spinaciaoleracea* (0.432 mg/L), *Amaranthus tricolor* (0.408mg/L) and *Amaranthus polygonoides* (0.288 mg/L).Sewage and tap water also has beyond level compared with the permissible limit of Copper concentration. (Figure 1.2). Zinc concentration in *Amaranthusaritis,Alternantherasessilis, Spinaciaoleracea, Amaranthuspolygonoides, Amaranthus tricolor*, sewage water, tap water were 0.241, 0.142, 0.154, 0.251, 0.262, 0.240, 0.249 mg / L respectively (Figure 2.1).

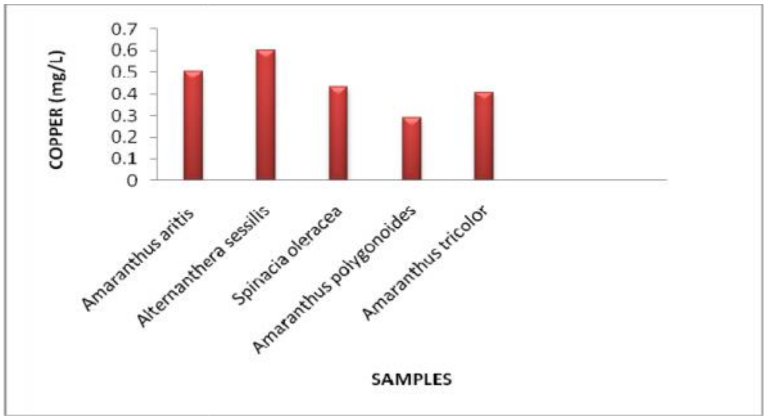


FIGURE 1.1: Copper level in spinach samples (permissible limit- 0.05 mg/L)

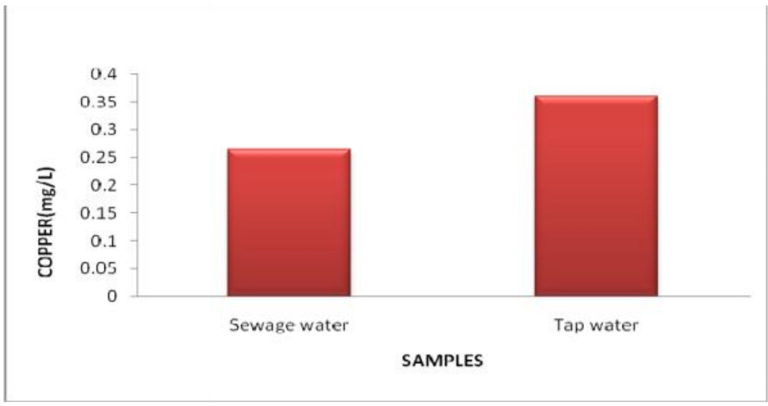


FIGURE 1.2: Copper level in water samples (permissible limit- 0.05 mg/L)

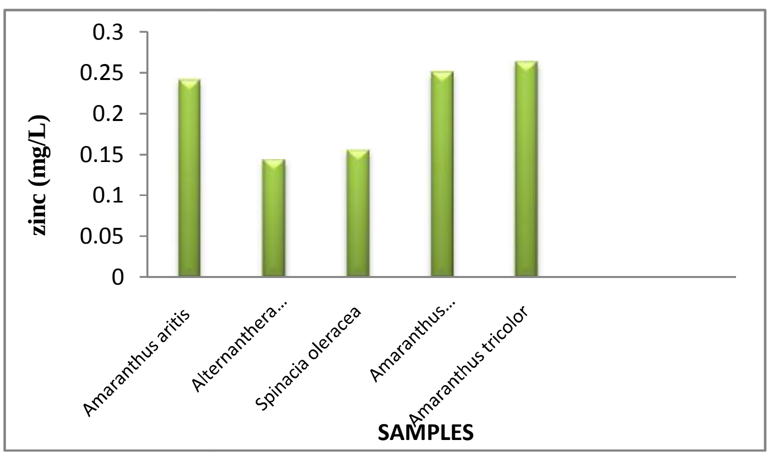


FIGURE 2.1 Zinc level in plant samples (permissible limit- 5.0 mg/L)

Zinc metal was found to be at the lower permissible limit (5 mg / L) for the five plant species. Hence, *Amaranthus tricolor* showed highest intake of Zinc (0.262) and *Alternanthera sessilis* exhibited trace amount of Zinc (0.142). Effects of Zinc in human beings are diarrhea, abdominal problems and high intake of Zinc concentration prevents our body from absorbing some antibiotic medicines (Linus Pauling Institute). Therefore, selected plant and water samples have below the permissible limit of Zinc concentration hence these plants species has no Zinc effect. Sewage and Tap water samples has trace amount Zinc concentration (Figure 2.2). Cadmium concentration was not found in *Amaranthusaritis, Alternanthera sessilis, Spinaciaoleracea, Amaranthus polygonoides*, *Amaranthus tricolor* plant species and also in Sewage and Tap water samples. Cadmium is very toxic to living organism mainly human beings. Chandran *et al* (2012) reported, concentration of Cadmium in *Alternanthera sessilis* was 2.65 mg/L in same study area. After 2012, the Cadmium concentration was drastically decreased which means there was no accumulation of Cadmium in those plant and water samples. Cadmium concentration was not found in both sewage and tap water samples.

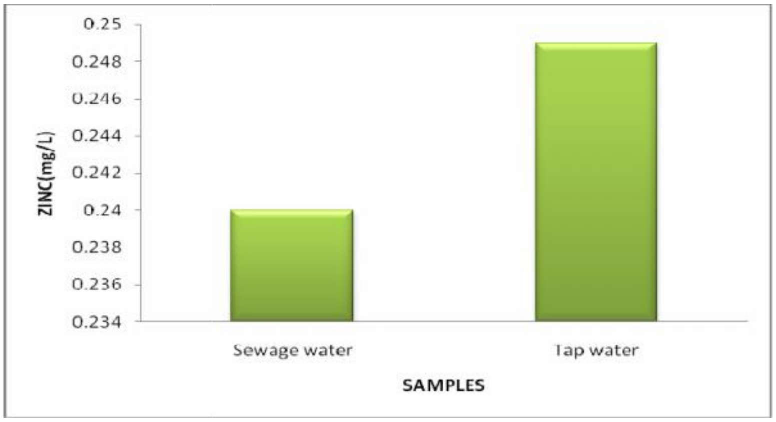


FIGURE 2.2: Zinc level in water samples (permissible limit- 5.0 mg/L)

ACKNOWLEDGMENT

Authors gratefully acknowledge to MHRD, Government of India, New Delhi for providing financial support and establishment of National Centre of Excellence in Statistical and Mathematical Modeling on Bioresources Management in Thiagarajar College, Madurai (F. No. 5-6/2013 -TS-VII). T. Kubendran thank to Director, ZSI, Kolkata and Dr. Avtar Kaur Sidhu, Scientist D & Officer-in-Charge, HARC, ZSI Solan for constant support and encouragement for constructive criticism in preparation of this manuscript.

REFERENCES

Baker, A. J. M., McGrath, S. P., Sidoli C.M. D. and Reeves, R.D. (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resources, Conservation and Recycling.* **11**, 41-49.

Balachandran C & Ramachandra, T.V. (2011) Distribution and Biology of the Mayflies (Ephemeroptera) of Western Ghats. *Sahyadrie News.* **35**,1-20.

Brooks, R.R. (1998) Plants that hyperaccumulate heavy metals, their role in phytoremediation, microbiology, archeology, mineral exploration and phytomining, *CAB Internat,* New York.

Cappuccino, G.J and Sherman, N. (2005) Microbiology- A Laboratory Manual, VII Edition, *Pearson Education India.*

Center, T.D., Hill, M.P., Cordo, H. and Julien, M.H. (2002) Water hyacinth. In: Van Driesche, R. Biological Control of Invasive Plants in the Eastern United States. *USDA Forest Service Publication FHTET-2002.* **04**, 41-64.

Chandran, S., Niranjana, V. and Joseph, B. (2012) Accumulation of Heavy Metals in wastewater irrigated crops in Madurai, India .*Journal of Environmental Research and Development,* **6**(3), 432-438

Chary, N.S., Kamala, C.T. and Raj, D.S. (2008) Assessing risk of heavy metals from consuming food grown on sewage irrigated soil and food chain transfer. *Journal of Ecotoxicology and Environmental safety,* **69**(3):513-524.

Deram, A., Petit, D., Robinson, B., Brooks, R.R. and Gregg, P. (2000) Natural and indused heavy-metal accumulation by *Arrhenatherumelatius*: Implications for phytoremediation. *Common Soil Sci. Plant Anal,* **31**, 413-421.

Edwin, G.A., Gopalsamy, P. & Muthu, N. (2014) Characterization of domestic gray water from point source to determine the potential for urban residential reuse: a short review. *Appl Water Sci* **4**, 39–49.

Ferner, D. J. (2001) Toxicity, heavy metals, *e Med J,* 2(5), 1.

Gowrek, B. (1992) Lead inactivation in soil by zeolites. *Plant soil,* **143**, 71-74.

Hamilton, E.I. (1995) State of art of trace element determinations in plant matrices: determination of chemical elements in plant matrices, an overview. *Sci Total Environ*, **176**, 3-14.

Jaikumar, M. (2012) A review on water hyacinth (*Eichhornia crassipes*) and phytoremediation to treat aqua pollution in Velachery Lake, Chennai–Tamil Nadu. *International Journal of Recent Scientific Research*, **3**(2) 95 – 102.

Jarupl, V. (2003) Hazards of Heavy metal contamination, *Br Med Bull*, **68**(1), 167-182.

Kadam, A.M., Nemade, P.D., Oza, G.H. and Shankar. H.S. (2009) Treatment of municipal wastewater using laterite-based constructed soil filter. *Ecol Eng.* **35**, 1051–1061.

Kadam, A.M, Oza, G.H., Nemade, P.D. and Shankar, H.S. (2008) Pathogen removal from municipal wastewater in constructed soil filter. *Ecol Eng.* **33**, 37–44.

Khan, S., NazAsim, A., Ahmad, S.S., Yousaf, S. and Muhammad, S. (2013). Toxicity and bioaccumulation of heavy metals in spinach seedlings grown on freshly contaminated soil. *Pak. J. Bot.,* **45**(S1):501-508.

Kubendran T. and Ramesh, M. (2016) Composition and distribution of aquatic insect communities in relation to water quality in two freshwater streams of southern Western Ghats, India. *Journal of Entomology and Zoological Studies*. **4**(5), 689-695.

Kubendran T. and M. Ramesh (2016) Monitoring of adult aquatic insect activities and ecological role in the riparian zone of streams in the Western Ghats, India. *IRA-International Journal of Applied Science.* 4(3): 460-470.

Kubendran, T., C. Selvakumar, A. K. Sidhu., S.M. Krishnan and A. Nair (2017) Diversity and distribution of Baetidae (Insecta: Ephemeroptera) larvae of streams and Rivers of the southern Western Ghats, India. *Journal of Entomology and Zoology Studies*. **5**(3), 613-625.

Kubendran, T., C. Selvakumar, A. K. Sidhu., A. Nair and S.M. Krishnan (2017) Baetidae (Ephemeroptera: Insecta) as Biological Indicators of Environmental Degradation in Tamiraparani and Vaigai River Basins of Southern Western Ghats, India. *Int.J.Curr.Microbiol.App.Sci.* **6**(6), 558-572.

Lehoczky, E., P. Marth and I. Szbados (2000) The Cadmium uptake by lettuce on contaminated soil as influenced by liming.*Common Soil Sci Anal*, **31**, 2433-2438.

Mosby, C. V., W. D. Glanze and K. N. Anderson (1996) Mosby Medical Encyclopedia, *The Signet:* Revised edition. St. Louis. **22**, 25-21.

Muralikrishnan, S., N.A. Nagendran., D. Pandiaraja., A Nair and T. Kubendran (2017) Avifaunal Diversity and Water Quality Analysis of an Inland Pond, Kondagai Village, Sivaganga District, South India. Int.J.Curr.Microbiol.App.Sci., **6**(7), 4437-4452.

Nalawade, P.M., A.D. Bholay and M.B. Mule (2015) Studies on Assessment of Heavy Metals in Samples Collected from Surrounding Area of Fly Ash Dumping Ground, *Universal Journal of Environmental Research and Technology,* **5** (3), 131-140.

Nemeth, T., Molnar, E., Csillag, J., Bujtas, K. and Partay, G. 1993. Fate and plant uptake of heavy metals in soil-plant system studied on soil monoliths. *AgrokemiaesTalajtan,* 42:195-207.

Rattan, R.K., S.P. Datta., P.K. Chhonkar., K. Suribabu and A.K. Singh (2005) Long term Impact of irrigation with sewage effluents on Heavy metal content in soils, crop and groundwater-A case study. *Agriculture, ecosystems and Environment.* **109**, 310-322.

Trivedy, R.K. and P.K. Goel (1986) Chemical and Biological Methods for Water Pollution studies, *Environmental Publication.* **6**, 132-144.

U.S. EPA (1988) Design Manual Constructed Wetlands and Aquatic Systems for Municipal Wastewater Treatment. U.S. Environmental Protection Agency. Report no. EPA/625/1- 88/022. Office of Research and Development, Cincinnati, OH, 83.

UN Report (2003) Water for people, water for life. World Water Development Report (WWDR). Unesco And Berghahn Books.

Wang, Y and M. Greger (2004) Clonal differences in mercury tolerance, accumulation and distribution in willow. *Journal of Environmental Quality,* **33**, 1779-1785.