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Annexure – VII
UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

**PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE
FINAL REPORT OF THE WORK DONE ON THE PROJECT**

- 1. Title of the Project** : Metal hyper accumulation studies in some leafy vegetables.
- 2. Name and address of the Principal Investigator:** Mrs. Sujata Gaherwar, A-18,
Suryavihar, Junwani, Bhilai-
490020
- 3. Name and address of the Institution:** Dept. Of Botany, Shri Shankaracharya
Mahavidyalaya Junwani, Bhilai.
- 4. UGC approval no. and date** : F. MS-41/202081/XII/14-15/CRO Bhopal dated
08-06-2015
- 5. Date of implementation** : 19-06-2015
- 6. Tenure of the project** : Two years (19/06/2015 – 19/06/2017)
- 7. Total grant allocated** : 4,00,000/- (Four lakhs only)
- 8. Total grant received** : 2,40,000/- (Two lakhs forty thousand only)
- 9. Final expenditure** : 4,02346/- (Four lakhs two thousand three hundred
and forty six)
- 10. Title of the project** : “**Metal hyper accumulation studies in some leafy
vegetables.**”
- 11. Objectives of the project** : Study was made with the following objectives -
 - To find out contamination level of heavy metals in green leafy vegetables.
 - Determination of maximum permissible limit and minimum toxic limit of each test metal in selected study plants.
 - To observe and report MCL of HMs in leafy vegetables grown in conditions simulating the contaminated land with effluent discharge.
 - Reporting of the findings would help us know that actually how much amount of toxic HMs could be present in healthy appearing GLVs.
 - The outcome of the project should be beneficial to society.

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12. Whether objectives were achieved: Yes,

- In the project studies were made on *Coriandrum sativum*, *Spinacea oleracea* and *Trigonella foenum-graecum* and they were found to accumulate heavy metals viz. Chromium and Arsenic.
- Test metals were found to be accumulated in roots and leaves of the test plants.
- Chromium was found to accumulate most in all the three test plants, surpassing the permissible limit of the test HMs, laid by Pollution control division (Indian standards).
- The findings of the project are beneficial to society, as many people otherwise do not know the consequences of watering the plants with not so clean water / waste water, even from effluent discharge.
- Students, staff in the college and also others in contact are made aware that the test plants i.e. *Coriandrum sativum*, *Spinacea oleracea* and *Trigonella foenum-graecum* are more sensitive to some test metals ie. Arsenic and Chromium, and therefore should be grown in clean, effluent free irrigating water.
- GLVs showing extraneous growth, should not be eaten, as it is the result of contamination of HMs.

13. Achievements from the project :

- A survey of sites of growing vegetables in Bhilai was made, and the sites being irrigated with contaminated / effluent discharge water could be identified.
- The green leafy vegetables being used in abundance, and which could be easily grown in simulated condition were identified as *Coriandrum sativum*, *Spinacea oleracea* and *Trigonella foenum-graecum*.
- Methods for detection of test metals viz. Arsenic, Lead, Mercury, Chromium and Selenium were standardised..
- Percentage absorption of test metals by test plants was studied and the extent, to which contamination can be caused by them, could be reported.

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- Comparative analysis could be made between the HM absorption by the three plants.
- Comparative analysis could also be made between the rates of absorption of the test metals by the test plants.
- The benefit of using bio-fertilizers could be observed / proven.
- The more we make the use of green manure, bio-fertilizers, bio-insecticides and compost, the more improvement in soil condition is achieved.
- The use of compost and green manure effectively act as the barrier, preventing the entry of HMs into the plants'
- Whatever HMs could accumulate in plants, were restricted to much extent in the roots only, of test plants.
- Out of the five test metals studied, it is the chromium, to which the test plants responded most.

14. Summary of the findings : Separate sheet attached

(in 500 words)

15. Contribution to the society : The following reported findings could help the people of society to know that the -

- *Coriandrum sativum* (Dhaniya) and *Trigonella foenum-graecum* (Methi) are more susceptible to HMs than *Spinacea oleracea*, the latter could survive even at higher concentrations of test heavy metals, to which the formers could not sustain.
- People in contact, would be made aware of the conspicuous morphological changes in the leaves of the test plants, which are in the form of thick and darker leaves.
- Vegetable growers would be advised, not to use waste water for irrigation.
- They and people in general would be made aware that bio-fertilizers do not cause harm to the plants as well as the soil.
- The use of green manure along with compost should be used generously to avoid accumulation of HMs in the test plants or even in other plants in general.

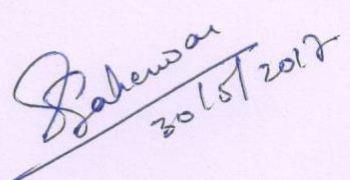
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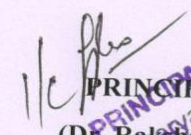
- Neutral or nearly neutral pH of the soil, also helps in restricting the HMs in the soil only.

16. Whether any PhD. enrolled/produced out of the project : The Principal Investigator is pursuing her Doctorate.

17. No. of publications out of the project : Three research papers communicated based on the results obtained from the work. Two are already published and one is in process. Details are mentioned below -

S. No.	Publication Details		
	Title of paper	Journal Details	Impact factor /NAAS Rating
1.	Estimation of chlorophyll content of some green leafy vegetables for their biochemical properties	<i>Indian Journal of Scientific Research</i> 13 (2): 170-171, 2017	ISSN:0976-2876 (Print) ISSN: 2250-0138 (Online) ISI IF – 1.928 (2017)
2.	Effect of heavy metals as environmental stress on some leafy vegetables	<i>Indian Journal of Scientific Research</i> 12 (2): 197-199, 2017	ISSN: 0976-2876 (Print) ISSN: 2250-0138(Online) ISI IF – 1.928 (2017)
3.	Estimation of chlorophyll content of some medicinal plants for their biochemical properties	Communicated in <i>Flora and Fauna</i>	


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SUMMARY

In general people consume lot of green leaves as vegetables or as condiments. In this way directly or indirectly chlorophyll contents play a major role in making us healthy. Green leafy vegetables (GLVs) as against chlorophyll are a rich source of minerals (including iron, calcium, potassium, and magnesium) and vitamins.

Looking at their nutritional value, people tend to eat them more. But looking at the present scenario, excessive use of chemical fertilizers, insecticides, pesticides etc., makes them easily contaminated with heavy metals (HMs). The edible part of these GLVs is leaves and tender stem, which can get easily affected by HMs present in chemicals as well as in irrigated water. Keeping this in mind the present work is taken up to study the maximum contamination level of test heavy metals, getting accumulated in them.

The GLVs selected for study are *Coriandrum sativum*, *Trigonella foenum-graecum* and *Spinacea oleracea*. The three plants are consumed in abundance.

Heavy metals can accumulate and migrate in soil environments. Due to their cumulative effects and long-term interactions, accumulation of heavy metals in soil negatively affects regional eco-safety and poses a threat to relevant animals and plants. Additionally, heavy metals can enter human bodies through the food chain, leading to an increased incidence of chronic diseases such as deformity and cancer.

In recent years, heavy metal contamination as a result of electroplating, chrome plating, leather tanning, textile dyeing, batteries, paints and waste, has become a serious problem all over the world. Heavy metal is a major threat to the environment, animals and humans due to their extreme toxicity.

The toxic metals taken are the ones included in the effluent discharges from Re-rolling mills, Steel Plant, rice mills and other industries. The effect of Arsenic (As), Lead (Pb), Mercury (Hg), Selenium (Se) and Chromium (Cr) was studied on test plants. In this context toxicity symptoms, accumulation effects and accumulation sites of test heavy metals were studied on test plants. While doing so maximum permissible

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limit and minimum toxic limit of each heavy metal (response dose) in selected study plants was observed. For quantitative analysis of each test heavy metal standard method given in Standard Methods for the Examination of Water and Wastewater (21st edition, APHA) is followed.

The experiment was performed using 13 x 13 polythene bags, filled with approximately four kg of soil. The seeds were sown and once the plants attained proper vegetative growth, after 20 -25 days, the heavy metal doses were given to them. Five replicates of each set were maintained. Observations were done regularly and constantly recorded. Experiments were performed to see effect of test heavy metals on test plants. This included qualitative as well as quantitative observations. Qualitative included total morphological changes, while quantitative was in terms of chlorophyll estimation as well as heavy metal inclusion in different plant parts. Both qualitative and quantitative parameters helped in studying dose response for each heavy metal, determination of maximum permissible limit and minimum toxic limit in selected study plants.

Proper mixing of clay with sand , FYM, compost, neem khali and a little of super phosphate, resulted in a proper combination, as certified by the soil testing laboratory. The combination was such that it didn't promote the intake of Pb, Hg and As , supporting the view of (Ghosh and Bhattacharya, 2004).

The magnitude of heavy metals detected in the studied leafy vegetables can be arranged as $Cr > As > Pb$. Chromium was found to accumulate most in all the three test plants, surpassing the permissible limit of the test HMs, laid by Pollution control division (Indian standards). While Hg and Se had no effect at all on the *Coriandrum sativum*, *Spinacea oleracea* and *Trigonella foenum- graecum*.

Coriandrum sativum (Dhaniya) and *Trigonella foenum-graecum* (Methi) are more susceptible to HMs than *Spinacea oleracea*, the latter could survive even at higher concentrations of test heavy metals, to which the formers could not sustain.

The benefit of using bio-fertilizers could be observed / proven. The more we make the use of green manure, bio-fertilizers, bio-insecticides and compost, the more improvement in soil condition is achieved. The use of compost and green manure

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effectively act as the barrier, preventing the entry of HMs into the plants. Whatever HMs could accumulate in plants, were restricted to much extent in the roots only, of test plants. Neutral or nearly neutral pH of the soil also helps in restricting the HMs in the soil only.

The HMs got accumulated more in roots, than in the leaves of all the three test plants. Though this could have been a sign of relief, if it would have been within the limits set by WHO or by European standards. But the amounts of test HMs were found well above them. The maximum contamination level of As is 0.05 mg / l, of lead is 15 µl, of Hg is 2 µl, Cr is 100 µl and of Se is 50 µl. looking at the values of MCL of all test plants in Table 4, it is clear that the concentrations of studied test metals are above the standard permissible levels thus might be in concern for the human consumers.

EFFECT OF HEAVY METALS AS ENVIRONMENTAL STRESS ON SOME LEAFY VEGETABLES

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ABSTRACT

Under present investigation effect of Arsenic (As), Chromium (Cr), Lead (Pb), Mercury (Hg) and Selenium (Se) was observed on *Coriandrum sativum* and *Spinacea oleracea*. From the qualitative morphological studies it was observed that *Coriandrum sativum* could survive the most, the toxic effects of the concentration doses of test heavy metals given.

KEYWORDS: *Coriandrum sativum*, *Spinacea oleracea*.

The heavy metals keep accumulating in our food items through various kinds of pollution and because of this when they reach the end of food chain i.e. in human body; appears in the form of various ailments. These may vary from simple allergies to severe neurological disorders.

The present study is to see the accumulation level and sites of deposition of Arsenic (As), Chromium (Cr), Lead (Pb), Mercury (Hg) and Selenium (Se) in *Coriandrum sativum* L. and *Spinacea oleracea* L.; the most commonly used green leafy vegetables. During the preliminary studies the effect of heavy metals is observed as morphological disparity in test plants in comparison to the control. This is then taken into account for further studies of heavy metals to achieve concise results.

MATERIALS AND METHODS

The test plants are grown in polythene bags having approximately four kgs. of soil. The seeds of the test plants are sown and doses of known concentrations of HMs are given to five replicates per set of experiment; as per the research design.

The techniques used for detection of HM concentrations in test plants are taken from the Standard Methods, published by American Public Health Association (APHA, 21st edition).

The chemicals, reagents used are of AR grade. Borosilicate glassware is used for performing different tests.

Qualitative morphological observations were made to see the effect of HMs on test plants, which included overall growth and development of the plants. For reporting the sites of accumulation of

HMs, sample drying in oven is followed by acid digestion (HNO₃: H₂SO₄: 2:1). These pretreated samples are then proceeded for spectrophotometric studies.

A Systronic made spectrophotometer was used for concentration analysis studies.

RESULTS AND DISCUSSION

HM doses were given to test plants after 20- 25 days of sowing seeds. The concentrations given were of 20, 30, 40 and 50 ppm of test HMs. Qualitative morphological observations were made every 10 days after treatment. The observations were as mentioned in the table.

From the above observations of Selenium and Mercury, samples were collected from their test plants and after acid digestion spectrophotometric readings showed no accumulation of above HMs in their above ground as well as underground parts. Though leafy vegetables such as *Amaranthus hybridus*, *Amaranthus sp.*, *Cucurbita maxima*, *Ipomoea batatas*, *Solanum villosum*, *Solanum scabrum*, and *Vigna unguiculata* were explored for their capabilities to accumulate selenium by Petro et al., 2015.

Lead was seen to affect the test plants even at lower (20 -30 ppm) concentrations and more damage signs were seen over the prolonged time period, after the treatment of the doses. Lead is non-essential toxic element which causes carcinogenic effects in human, even at very low concentrations (Nazar et al., 2012). Domergue and Vedy (1992) reported that, when Pb is absorbed from the soil by crops, it remains mainly in the root area as it cannot effectively go through the endodermis of roots.

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Leaves, however, can absorb vast quantities of Pb from the atmosphere. Hence, GLV have the capability to accumulate Pb from the soil as well as from the atmosphere through their leaves. Lead has

been reported as a severe cumulative body toxin which enters the body through food, air and water and cannot be eliminated by washing the vegetables (Abbas et al., 2010 and Zamor et al., 2012).

Table 1: Qualitative morphological effects of Test Heavy Metals on Test Plants over different incubation periods.

HMs	Concn. Doses of HMs	<i>Coriandrum sativum</i> L.			<i>Spinacea oleracea</i> L.		
		10 DAT	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
Lead (Pb)	20 and 30 ppm.	No change from control	No change	Wilting occurred	No change	Wilting occurred	
	40 and 50 ppm.	No change	Slight stunted growth	Plants seemed unhealthy	Yellowing of leaves along with smaller size	Stunted growth along with wilting	Plants looked weak and wilted
Arsenic (As)	20 and 30 ppm.	No change	No change	No change	No change	No change	Wilted plants
	40 and 50 ppm.	No change	No change	Yellowing of leaves	No change	Yellowing of leaves with wilting and stunted growth	
Chromium (Cr)	20 and 30 ppm.	Normal growth as of control			Normal growth as of control		
	40 and 50 ppm.	No change	No change	Flowering occurred later than control	No change	Yellowing of leaves and stunted growth	Growth is highly diminished
Mercury (Hg)	20,30,40 and 50 ppm	No changes from the control			No changes from the control		
Selenium (Se)	20,30,40 and 50 ppm	No changes from the control			No changes from the control		

From the qualitative morphological effects of arsenic and chromium on *Coriandrum sativum* and *Spinacea oleracea* arsenic caused more damage to test plants than chromium and *Coriandrum sativum* could resist more to environmental stress due to heavy metal presence than *Spinacea oleracea*.

ACKNOWLEDGEMENT

The first author is thankful to UGC-CRO (Bhopal) for providing the financial support to undertake the work.

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ESTIMATION OF CHLOROPHYLL CONTENT OF SOME GREEN LEAFY VEGETABLES FOR THEIR BIOCHEMICAL PROPERTIES

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ABSTRACT

Chlorophyll is a green pigment, that's instrumental in photosynthesis. Studies also suggest it's instrumental in promoting good health. Chlorophyll benefits the body in a unique and distinctive ways. It helps to cleanse harmful toxins from the body and it is also used to fight infection. A recommended and regular intake of chlorophyll can keep the circulatory and digestive system much healthier. Environmental pollutants such as toxic metals can quickly destroy health. Chlorophyll binds with toxic metals to hamper absorption, and studies have also shown it can do the same with some carcinogens. In the present study, the chlorophyll content was extracted from the leaves of twelve herbaceous plants which are being used as leafy vegetables. Observations were characterized by spectroscopy. Concentration of chlorophyll a and b was calculated using N, N-Dimethylformamide incubation method. *Coriandrum sativum* was identified as having maximum chlorophyll content (2.33mg/ gram of fresh weight of leaf) as compared to other test plants.

KEYWORDS: Chlorophyll a, Chlorophyll b, Leafy Vegetables, Spectroscopy

As we all know that leaf chlorophyll content is an important parameter for testing plant status. For example, chlorophyll content can be used as an index of the photosynthetic potential as well as of plant productivity (Carter, 1998; Filella et al., 1995). In addition, chlorophyll gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll (Filella et al., 1995). In recent years, chlorophylls, the most abundant pigments in green plants are gaining increasing importance in the human diet, not only as food colorants, but also as healthy food ingredients. (Xue and Yang, 2009).

Chlorophyll is a green pigment, which is structurally similar to porphyrin pigments such as heme and it is produced through the same metabolic pathway. Chlorophyll benefits the body in a unique and distinctive ways. It helps to cleanse harmful toxins from the body and it is also used to fight infection. A recommended and regular intake of chlorophyll can keep the circulatory and digestive systems much healthier.

In general people consume lot of green leaves as vegetables or as condiments. In this way directly or indirectly chlorophyll contents play a major role in making us healthy. Green leafy vegetables as against chlorophyll are a rich source of minerals (including iron, calcium, potassium, and magnesium) and vitamins, including vitamins K, C, E, and many of the B vitamins. They also provide a

variety of phytonutrients including beta-carotene, lutein and zeaxanthin, which protect our cells from damage and our eyes from age-related problems, among many other effects. Dark green leaves even contain small amounts of Omega-3 fats. Perhaps the star of these nutrients is Vitamin K. A cup of cooked greens provides at least nine times the minimum recommended intake of Vitamin K. Recent research has provided evidence that this vitamin may be even more important than we once thought, as it regulates blood clotting, protects bones from osteoporosis, help prevent atherosclerosis by reducing calcium in arterial plaques, protect us from arthritis, help prevent diabetes etc. So the more the chlorophyll content the more nutritious the leaves will be.

In the present study chlorophyll content of some common leafy vegetable plants was estimated and compared for their nutritional value in terms of amount of chlorophyll present in them.

MATERIALS AND METHODS

N, N-Dimethyl formamide incubation method (Inskeep and Bloom, 1985 with Annon, 1949) was followed for chlorophyll estimation of *Coriandrum sativum*, *Spinacea oleracea*, *Brassica campestris*, *Mentha arvensis*, *Allium cepa*, *Raphanus sativus*, *Brassica oleracea var. botrytis*, *Brassica oleracea var. capitata*, *Chenopodium album*, *Amaranthus tricolor*, *Alternanthera sp.* and *Trigonella foenum-graecum*.

¹Corresponding author

Analytical reagents used during the extraction process were of AR grade. Distilled water was used wherever needed. Quartz cuvette (1cm²) were used and corresponding solvent was taken as reference during spectrophotometric observation. Every procedure (for each plant sample and extracting solvent) was triplicated for maintaining the precision of analytical results.

A Systronic made spectrophotometer was used for chlorophyll estimation at 664.5nm and 647.4 nm.

RESULTS AND DISCUSSION

Healthy leaves of test plants were taken and after washing and drying, (under fan) chlorophyll estimation test was performed and the observations were tabulated.

Among the GLVs tested, *Coriandrum sativum* contained the highest amount of total chlorophyll. Next in the series were *Trigonella foenum-graecum*, closely followed by *Amaranthus tricolor*, *Mentha arvensis*, *Chenopodium album* and *Alternanthera sp.* The least chlorophyll content (among the tested GLVs) was observed in *Brassica oleracea var. capitata*, followed by *Allium cepa*.

The highest value of the ratio is reported in *Amaranthus tricolor* followed by *Brassica oleracea var. botrytis* indicating presence of large amount of chlorophyll in them. We can say that the leafy vegetables analysed in our study are the crops with a relatively high content of chlorophylls, similar to studies of Kopsell et al.2004. (Znidarcic et al.2011)

CONCLUSION

To maximize the chlorophyll intake in the body, one should wisely make use of available vegetables. A vegetable like broccoli—while richly green on the outside—tends to become more whitish toward the center of the stalk, and on a cup-for-cup basis, can be a less concentrated source of chlorophyll than a green leafy vegetable, which is not only thinner but also green "through and through."

As from the above result *Coriandrum sativum* and *Mentha arvensis* contain more chlorophyll, therefore, though as condiments, their small but frequent use would be beneficial for health.

At the same time the other above mentioned GLVs are recommended to be added to regular meals, in abundance.

ACKNOWLEDGEMENT

The first author is thankful to UGC-CRO (Bhopal) for providing the financial support to undertake the work.

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