S. G. V. C. Vidya Prasarak Trust's,
Matoshri Gangamma Veerappa Chiniwar Arts, Commerce & Science College,
MUDDEBIHAL-586212. Dist. Vijayapur (Karnataka)
(Accredited with CGPA of 2.58 on seven point scale at 'B' Grade)

✆ : 08356220329 / 222175
FAX : 08356220329 / 221121

* email : princmgvc@gmail.com *

Ref. No. : .... Date : 16.1.2020

COLLABORATION

M.G.V.C. ARTS, COMMERCE AND SCIENCE COLLEGE MUDDEBIHAL DIST BIJAPUR (DEPT OF BOTANY)

AND

BLDEA's ARTS, COMMERCE AND SCIENCE DEGREE COLLEGE JAMKHANDI

(DEPT OF BOTANY)

M.G.V.C.Arts,Commerce and Science College Muddebihal (Prof: S.V.Gurumath Associate Professor Department of Botany) and BLDEA's Arts,Commerce and Science College Jamkhandi (Dr. N.M.Rolli Associate Professor Department of Botany) wish to initiate collaboration in research and in other disciplines.

The main objective is to foster collaboration, exchange of information on research learning materials and other literature relevant to the educational and research programmes.

Pro f: S.V.Gurumath

Associate Professor

Department of Botany

Dr. N.M.Rolli

Associate Professor

Department of Botany

PRINCIPAL,
M. G. V. C. Arts, Com. & Science College
MUDDEBIHAL - 586212.

S. G. V. C. Vidya Prasarak Trust's,
Matoshri Gangamma Veerappa Chiniwar
Arts, Commerce & Science College,
MUDDEBIHAL-586212. Dist. Vijayapur (Karnataka)
(Accredited with CGPA of 2.58 on seven point scale at 'B+' Grade)
✆: 08356220329
FAX: 08356220329
* email : princmgvc@gmail.com * www.mgvcmbl.in *

Ref. No. : .... Date : ....

COLLABORATION

The collaboration is for research activity: Normally at college level, faculty require support of another institution where active research is being carried out. Against this backdrop, our faculty S.V. Gurumath, Department of Botany has formal agreement with Dr N M Rolli, Associate Professor BLDEA'S Arts, Commerce and Science College, Jamakhandi wish to initiate collaboration in research and other disciplines.

The main objective is to foster collaboration, exchange of information on research learning materials and other literature relevant to the educational and research programmes

Sl. No.	Author(s)	Title	Name of the Journal	Volume	Page Year
1	S.V.Gurumath, N.M.Rolli, Chanvan R R, Anantpur A.S. and Taranath.T. C	Heavy Metal Accumulation in Vegetables cultivated in agricultural soil irrigated with sewage and its Impact on Health	International Journal of Recent Scientific Research	Vol.11,Issue,04(C)	pp.38144-38150,April,2020
2	N M Rolli , S V Gurumath, M K Ganachari, B D Patil and S B Gadi	Zinc (Zn) accumulation and its Toxicity effects on Salvinia molesta Mitchell and Spriodela polyrhiza(L.)Schleid	A Web of Science Journal	Vol.2(1)	277-288 (2021)

PRINCIPAL,
M. G. V. C. Arts, Com. & Science College
MUDDEBIHAL - 586212.

M.G.V.C. Arts, Commerce & Science College MUDDEBIHAL -586212 Dt: Vijayapur.

web: www.biology-journal.org

ISSN: 0970-2
A web of Science Journal

Special Volume 2(1) : 2021

Indian Journal of Applied & Pure Biology

Academic Key words Impact Factor (2012) 3.0; Rated Journal 1.05

An International Research Journal of Biological Science















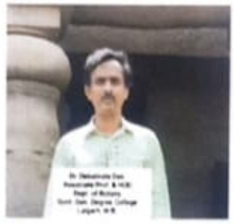
Papers based on 2 Day National e-Conference On Biodiversity Building and Natural Resource Management (NC BBNRM - 2021)

Date of Conference : 22nd & 23rd December, 2021
Date of Publication : 31st December, 2021

Abstracted & Indexed by Various National and International Agencies

ANKITA DUTTA
Research Scholar
Centre of Life Sciences
Vidyasagar University

Dr. Nandini Ghosh,
Assistant Professor
Department of Microbiology
Vidyasagar University



Dr. Mitu De, Associate
Professor, Department of
Botany, Gurudas College

V. Karthiya,Research Scholar,
Department of Botany,
Avinashilingam University,
Coimbatore-43

Indian J. Applied & Pure Bio. Special Vol. 2(1): 277-288 (2021). **ISSN: 0970-2091**
National e-Conference on Biodiversity Building and Natural Resource Management (NC BBNRM) Date: 22nd-23rd, December, 2021 **A web of Science Journal**

Zinc (Zn) accumulation and its Toxicity effect on *Salvinia molesta* Mitchell and *Spirodela polyrhiza* (L.) Schleid

[1]N.M. Rolli, [2]S.V. Gurumath, [3]M.K. Ganachari, [4]B.D. Patil and [5]S.B. Gadi

[1]BLDEA's Degree College Jamkhandi-587301 (India)
[2]MGVC Arts, Science and Commerce College, Muddebihal-586212 (India)
[3]Basaveshwar Science College, Bagalkot, Karnataka (India)
[4]Shri Jagadamba Degree College Hittinahalli L.T, (586215) Karnataka, (India)
[5]JSS College Dharwad, Karnataka, (India)

Corresponding author:
Dr. N.M. Rolli
Associate Professor BLDEA's Degree College, Jamkhandi, 587301
(Karnataka) (India)
E-mail: drnmrolli@rediffmail.com
Mob: 094488 96839

Abstract

Due to increasing awareness of toxic heavy metals contamination to the environment, studies of metal accumulation from the view point of metal removal from the contaminated water have been performed. The use of biological systems for removing metals from metal solution has the potential to achieve greater performance at lower cost. This is an emerging biological application based on 'Green Liver Concept' and operates on the principles of biogeochemical cycling.

The present study focuses on zinc toxicity on morphology and some biochemical parameters of *Salvinia molesta* Mitchell and *Spirodela polyrhiza* (L.) Schleid. The laboratory experiments were conducted for the assessment of morphological index parameters (MIP), biochemical parameters and metal (Zn) accumulation profile in test plants at various concentrations (viz. 5,10, 20, 30 and 40 ppm *Salvinia* and *Spirodela* at regular interval for 12 days. *Salvinia* and *Spirodela* shows visible symptoms like withering of roots, chlorosis and necrosis at higher concentration. With respect to *Spirodela* at higher concentration the lower surface of leaf turns pink to whitish. However, the test plants showed normal growth at lower concentration (5.0 ppm). The estimation of biochemical parameters *i.e* total chlorophyll, protein and carbohydrate of test plants showed a significant increase at lower concentration (5 ppm) of zinc and showed significant decrease with increase in exposure concentration and duration. Metal accumulation by test plants was maximum at 4 days exposure duration and marginal at subsequent concentrations and exposure duration. With respect to biochemical parameters the concentrations are significant. However, metal accumulation is significant at different concentrations and exposure duration.

Heavy metal pollution is a major environmental problem facing the modern world[8,36]. The global heavy metal pollution is increasing in the environment due to increase of human activities. However, it is gaining importance day by day due to its obvious effect on human health through the food chain[18]. Due to the increasing awareness of toxic heavy metals contamination to the environment, studies of metal accumulation from the view point of metal removal from the contaminated water have been performed[4,6]. Conventional methods including precipitation, oxidation, reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and evaporation recovery are expensive or ineffective[33]. The use of biological systems for removing metals from low metal solution (1 to 100 mg/g) has the potential to achieve greater performance at lower cost.

Aquatic plants and / or algae are known to accumulate metals and other toxic elements from contaminated water[34]. The bio-removal process using aquatic plants often exhibits two staged uptake process: on initial fast, reversible, metal binding process (biosorption) followed by a slow, irreversible, ion sequestration step (bioaccumulation). The initial metal biosorption by different parts of cells can occur via complexation coordination, chelation of metals, ion exchange, adsorption and micro precipitation. The bioaccumulation process is an active mode of metal accumulation by living cells[4,22]. This process is dependent on the metabolic activities of the cell, which inturn can be affected by the presence of metallic ions[33]. The accumulation of metals at higher concentration causes retardation of growth, biochemical activities and also generation of –SH groups containing enzymes.

The main goal of this study was to determine the performance of the test plants i.e *Salvinia molesta* and *Spirodela polyrhiza* to the different concentrations of Zinc (Zn) on morphology, biochemical constituents and accumulation of metal profile from the experimental pond under laboratory conditions.

Salvinia and *Spirodela*, free floating aquatic plants for unpolluted water bodies is maintained in a cement pots (1 meter diameter) under conditions at a temperature 28-30°C. About 20 g of young healthy *Salvinia* and *Spirodela* were acclimatized for two weeks in Arnon and Hoagland nutrient solution maintaining pH between 7.1-7.4. The concentrations of Zinc in the polluted water are in the range of 5, 10, 20, 30 and 40.0 mg/l and tap water as a control. Morphological Index Parameters (MIP) viz, root length, leaf length and breadth were observed for 12 days at interval of 4 days. Photographs of *Salvinia* and *Spirodela* which were taken by using

Canon's Power Shot G_2 digital camera were treated with different concentrations of zinc. For the further study the plants were harvested at the end of 4, 8 and 12 days exposure and are thoroughly washed with distilled water and used for the estimation of total chlorophyll, protein and carbohydrate and also for morphological observations. Plants harvested after 48 hrs were dried at 80^0C for 2 days for metal extraction.

The fresh test plant samples of 1 g is macerated in 100 ml of 80% (v/v) chilled acetone by using pestle and mortar. The centrifuged and supernatant was used for the estimation of total chlorophyll by standard Arnon method using 652 nm against the solvent (80% acetone as a blank)[12]. The protein was estimated by Lowry's method[7] using Bovine Serum Albumin (BSA) as a standard, using 660 nm and carbohydrates by phenol sulphuric acid method (Dubois) using glucose as standard at 490 nm[1]. Morphological characters were identified with the help of photographs, using Canon's Power Shot G_2 digital camera.

The estimation of metal Zn in the test plant was carried out by using standard method[23]. The dried and powdered 1 g plant material was digested by using mixed acid digestion method in Gerhardt digestion unit. The digested samples were diluted with double distilled water and filtered through Whatman filter paper No. 44. The estimation of Zn was done by atomic absorption spectrophotometer (AAS) (GBC 932 Plus Austrelia) with air acetylene oxidizing flame and metal hollow cathode lamp at 217.00 nm wavelength. Working standards (SISCOP-CHEM-Bombay Lab) were used for the calibration of instrument.

Statistical Analysis :

Data are presented as mean values ± SE from two independent experiments with three replicates each. Data were subjected to Two – way ANOVA to know significance between concentrations and between exposure duration for the accumulation of heavy metal, (Zn). Further, Dunet's test is also applied for multiple comparisons between control and other concentrations. Two – way ANOVA test also extended to know the significance between concentration and duration for biochemical parameters.

Toxic effect of Zinc on morphology :

The test plant showed luxuriant growth, shows increase in the lamina and breadth at low concentration (5.0ppm) in both test plants. In *Salvinia* at (5.0 ppm) of zinc was found to promote the laminal length by 1.48 ± 0.020 cm, 1.5 ± 0.04 cm, 1.666 ± 0.14cm and breadth by 1.6 ± 0.169cm, 1.666 ± 0.14cm and 1.7 ± 0.027cm at 4, 8 and 12 days exposure duration. Similarly root length by 2.61 ± 0.216 cm, 2.2 ± 0.205 cm and 2.4 ± 0.216 cm. In *Spirodela* at the lower concentration (5.0 ppm) showed increase in laminal length by 0.866 ± 0.027 cm, 1.0 ± 0.01cm and 1.1 ± 0.072cm and breadth by 0.633 ± 0.072 cm, 0.666 ± 0.054 cm and 0.766 ± 0.054 cm at 4, 8 and 12 days exposure duration respectively. Similarly the root length by 2.766 ± 0.144cm, 2.866 ± 0.165cm and 3.0 ± 0.216cm at 4, 8 and 12 days exposure duration respectively.

Morphometric assay is one of the quantitative tool for the assessment of toxicants measured by using Morphological Index Parameter (MIP). The rate of inhibition in the root and leaf (Fronds) is directly proportional to the concentration of zinc and in both test plants. Two way ANOVA test states that the concentrations are significantly toxic at 5% level but duration is not significant. MCA test also represents maximum deviation is at higher concentration compared to control. Both the test plants showed normal growth at their respective lower concentration (5.0 ppm).

However, in *Salvinia* at 40 ppm concentration shows severe inhibition of laminal length by 1.1 ± 0.081 cm, 1.033 ± 0.072cm and 0.933 ± 0.081cm and laminal breadth by 1.0 ± 0.08 cm, 0.966 ± 0.072 cm and 0.911 ± 0.081 cm at 4, 8 and 12 days exposure duration. Similarly the root length inhibition was noticed to 0.933 ± 0.222 cm, 0.9 ± 0.047 cm and 0.8 ± 0.216 cm at 4, 8 and 12 days exposure duration respectively. Similarly, in *Spirodela* also at 40 ppm concentration shows severe inhibition of laminal length by 0.600 ± 0.047 cm, 0.533 ± 0.027 cm and 0.366 ± 0.027 cm and laminal breadth by 0.60 ± 0.047 cm, 0.466 ± 0.027 cm and 0.311 ± 0.027 cm at 4, 8 and 12 days exposure duration. Similarly the test plant (*Spirodela*) also exhibited root inhibition by 1.566 ± 0.098 cm, 1.10 ± 0.047 cm and 0.80 ± 0.216 cm at 4, 8 and 12 days exposure duration (Tables-1 and 2).

The higher concentration of Zn (10 ppm to 40 ppm) exhibited toxicity symptoms like chlorosis, fall of leaves were observed about particularly after 8 days to 12 days exposure brownish marks were observed in *Salvinia*, however, in *Spirodela* the plants have lost their roots, leaves turned green to yellow on the upper surface, lower pink colour tends to creamy white in colour. Our results of toxicity symptoms of Zn at higher concentrations observed and were similar to Saygideger[20]. Sobero confirmed elongation of root in some members of Lemnaceae at different concentration of Zn[14].

Toxicity effect of Zn on Biochemical parameters :

The chlorophyll content was very sensitive to heavy metal toxicity. The results found that at 5 ppm of Zn is found to augment chlorophyll synthesis and was directly proportional to the concentration and exposure duration in both test plants. The chlorophyll of *Salvinia* increased by 3.08% (0.701 mg/g), 4.62% (0.724 mg/g) and 5.22% (0.725 mg/g) respectively at 4, 8 and 12 days exposure duration to 5 ppm Zn in comparison to control. The chlorophyll content in *Spirodela* increased with increase in the exposure duration at an exposure concentration of 5 ppm Zn to 0.721 mg/g, 0.752 mg/g and 0.806 mg/g respectively at 4, 8 and 12 days exposure duration (Tables 3 and 4).

A number of heavy metals are required by the plants as micronutrients[20] and they act as a cofactors of enzymes as part of prosthetic groups and involved in a wide variety of metabolic pathways, but higher concentration of heavy metals are toxic to plants[2]. Heavy metals in ecosystem induces physiological and genetical changes in plants[5]. The zinc is one of the important nutrient for various metabolic processes and required in minute quantities[24].

It is evident from the data that the lower concentration of 5 ppm stimulates chlorophyll synthesis. Pandey reported the stimulation of chlorophyll synthesis at lower concentration of Nickel in *Spirodela*[26]. The 5.0 ppm of Zn promotes the chlorophyll synthesis from 0.701 mg/g to 0.745 mg/g in *Salvinia* and 0.721 mg/g to 0.806 mg/g in *Spirodela* during 4 and 12 days. The enhancement percentage of chlorophyll at 12 days exposure is 5.22% in *Salvinia*, 15.14% in *Spirodela* when compared to its respective controls. Choudhary and Ramachandra observed stimulatory effect of 1.5 mg/lt Zn on *Nostoc muscorum* including chlorophyll, carbohydrate and protein content[15]. Plant possess unique ability to evolve tolerance by the induction of phytochelatin (PC)[5,16].

However, the higher concentration of Zn found to inhibit the chlorophyll synthesis in both test plants. The present inhibition is 12.5% (0.595 mg/g), 23.26% (0.529 mg/g) and 42.1% (0.410 mg/g) at 40 ppm concentration respectively at 4, 8 and 12 days exposure duration respectively in comparison to control in *Salvinia*. Similarly *Spirodela* also shows inhibitory effect at 40 ppm of zinc towards chlorophyll synthesis by 1.57% (0.628mg/g), 19.18% (0.590 mg/g) and 41.88% (0.465 mg/g) respectively at 4, 8 and 12 days exposure duration. The severity of chlorophyll inhibition increases with increase in the duration of exposure. The percent inhibition of chlorophyll at 12 days exposure is 12.1% in *Salvinia* and 41.88% in *Spirodela* when compared to their respective control. Van Asche and Clijsters reported the chlorophyll biosynthesis is attributed to the interaction of metals with –SH group involved in catalytic activity[2]. Algal cultures exposure to higher concentrations of Zn exhibited chlorosis and cell lysis. The degradation of light harvesting pigments might be attributed to the disruption of thylakoid membranes within the cell[3]. The inhibition of photosynthesis is mainly because of proteolytic PC halide reductase complex and the synthesis of δ amino levulinic acid (ALA)[30].

Two way ANOVA represents biochemical toxicity to the test plants, concentrations were significant at $P>0.01$ level but duration is not significant. (Tables 3 and 4).

The increase in carbohydrate content at 5 ppm, Zinc promotes the carbohydrate content by 52mg/m (18.3%), 66.0mg/m (24.52%) and 76.0mg/m (28.8%) respectively at 4,8 and 12 days exposure. In *Spirodela*, the carbohydrate synthesis is increased at 5 ppm exposure to 32.0 mg/m (6.66%), 38.0mg/m (11.76%) and 44.0mg/m (18.91%) respectively at 4,8 and 12 days in comparison.

The carbohydrates form an important organic constituent of the plant tissues. Our investigation showed that at lower concentrations of Zn (5ppm) enhances the carbohydrate synthesis from 52mg/m to 76mg/m in *Salvinia* and 32mg/m to 44mg/g in *Spirodela* from 4 to 12 days exposure respectively. The enhancements of carbohydrates at 12 days exposure is 28.8% in *Salvinia* and 18.91% in *Spirodela* when compared to their respective controls. Increased carbohydrate content in *Nostoc muscorum* at lower concentrations of Nickel and Magnesium observed[23]. Lower concentration of Zn activates the enzyme or is incorporated into metalloenzymes in electron transport system (ETS)[31].

Table-1. Effect of Zinc on morphology of *Salvinia molesta*

Conc-entr-ation (ppm)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control 0	1.966±0.19	1.9±0.216	1.91±0.216	1.4±0.080	1.366±0.788	1.4±0.080	1.433±0.027	1.511±0.027	1.501 ± 0.027
5	2.61±0.216	2.2±0.205	2.4±0.216	1.43±0.027	1.6±0.169	1.5±0.047	1.666±0.144	1.666±0.144	1.7±0.027
10	1.866±0.144	1.73±0.205	1.712±0.216	1.4±0.080	1.466±0.144	1.366±0.027	1.433±0.027	3.966±0.196	1.366±0.788
20	1.366±0.284	1.33±0.284	1.116±0.216	1.3±0.788	1.4±0.080	1.233±0.072	1.333±0.151	1.111±0.196	1.3±0.708
30	1.066±0.222	1.03±0.098	1.0±0.284	1.16±0.072	1.066±0.072	1.033±0.072	1.033±0.072	1.0±0.047	0.966±0.557
40	0.933±0.222	0.9±0.047	0.8±0.216	1.1±0.081	1.0±0.08	1.033±0.072	0.966±0.072	0.933±0.081	0.911±0.081

Values are expressed in cms

Mean values ± Standard Error

Table-2 . Effect of Zinc on morphology of *Spirodela polyrhiza*

Conc-entr-ation (ppm)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control 0	2.000±0.047	2.0±0.047	2.2±0.216	0.666±0.054	0.566 ± 0.054	0.733 ± 0.072	0.600 ± 0.0	0.733 ± 0.072	0.610 ± 0.272
5	2.766±0.144	2.866±0.165	3.0±0.216	0.866±0.027	0.633±0.072	1.0±0.0	0.666±0.054	±0.072	0.766±0.054
10	2.333±0.054	2.3±0.816	2.1±0.04	0.766±0.047	0.666±0.072	0.733±0.072	0.633±0.054	0.666±0.054	0.533±0.027
20	1.900±0.216	1.633±0.047	1.333±0.816	0.7±0.047	0.566±0.027	0.7±0.047	0.566±0.027	0.4±0.027	0.300±0.027
30	1.666±0.216	1.566±0.098	1.116±0.216	0.666±0.054	0.533±0.027	0.6±0.047	0.511±0.027	0.5±0.0	0.400±0.47
40	1.566±0.098	1.100±0.047	0.800±0.216	0.600±0.047	0.600±0.047	0.533±0.027	0.466±0.027	0.366±0.027	0.311±0.027

Values are expressed in cms

Mean values ± Standard Error

Table-3. Two way ANOVA for biochemical effects of Zn on *Salvinia molesta*

	Total chlorophyll	Protein	Carbohydrate
F-Value (between concentration)	10.027**	10.56**	6.787**
F-Value (between duration)	2.66	1.24	0.013

**Significant at P < 0.01 level

Table-4. Two way ANOVA for biochemical effects of Zn on *Spirodela polyrhiza*

	Total chlorophyll	Protein	Carbohydrate
F-Value (between concentration)	5.808**	23.84**	15.468**
F-Value (between duration)	0.488	0.249	0.045

**Significant at P < 0.01 level

Table-5 . Two way ANOVA with Dunet's test for multiple comparison for accumulation of Zn by aquatic macrophytes

	Salvinia	*Spirodela*
F-Value (between concentration)	5621.50**	2247.42
F-Value (between duration)	13.90**	6.723
Dunet's value	265.164	386.60
Control V/s 5.0 ppm	2690.41	2880.33
Control V/s 10.0 ppm	5202.50	5446.00
Control V/s 20.0 ppm	10768.08	11573.66
Control V/s 30.0 ppm	15645.00	13784.33
Control V/s 40.0 ppm	17804.50	16866.00

** Significant at P < 0.01 level

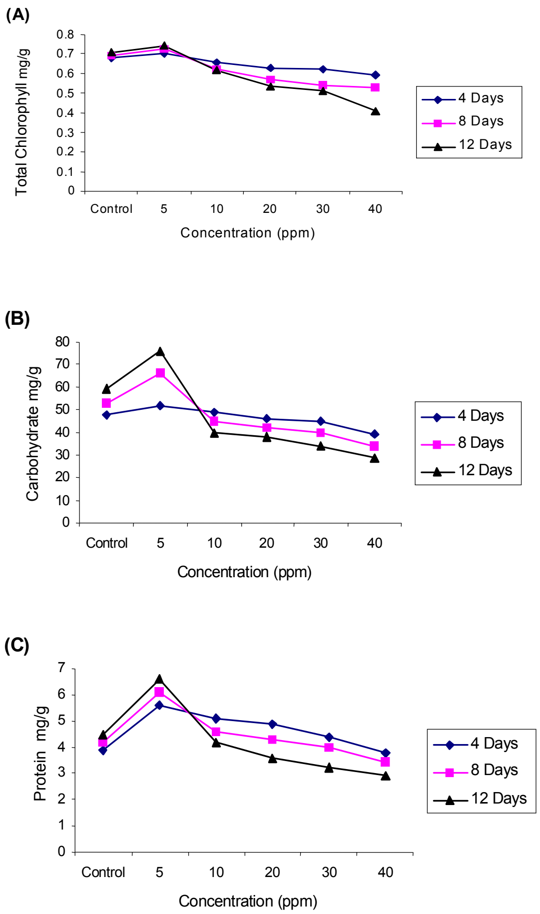


Fig: 1. Biochemical effects of Zinc on *Salvinia molesta*
(A) Total Chlorophyll (B) Carbohydrate
(C) Protein

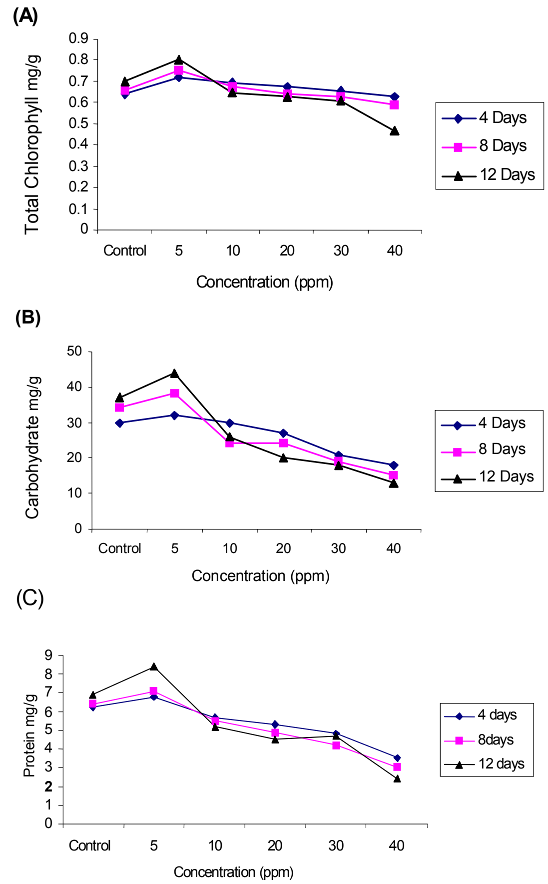


Fig: 2 . Biochemical effects of Zinc on *Spirodela polyrhiza*
(A) Total Chlorophyll (B) Carbohydrate
(C) Protein

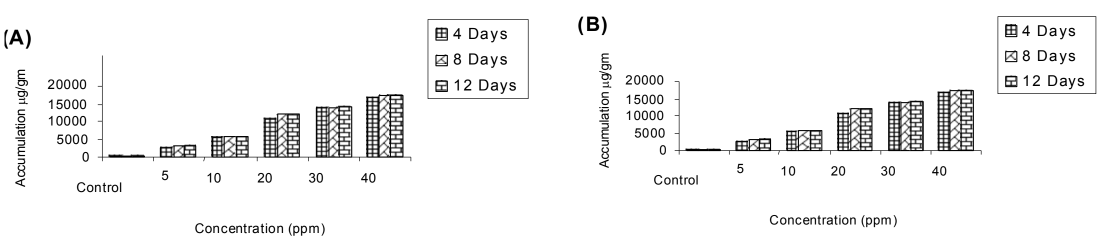


Fig: 3. Accumulation profile of Zinc by aquatic macrophytes
(A) *Salvinia* (B) *Spirodela*

However, the higher concentration of Zn 10, 20, 30 and 40 ppm of Zn, *Salvinia* was found to exhibit inhibition of carbohydrate synthesis. The 40 ppm Zn inhibited the synthesis of carbohydrate to 39.0mg/g, 34.0 mg/g and 29.0 mg/g respectively at 4, 8 and 12 days of exposure duration in comparison to control. In *Spirodela* at 40 ppm of Zn the inhibition of carbohydrate synthesis is 16.0 mg/g (46 %), 15 mg/g (55.88%) and 13.0 mg/g (64.87%) respectively at 4, 8 and 12 days exposure duration, the reduction in carbohydrate content can be attributed to the reduced rates of photochemical activities[17] and also Succinic Dehydrogenase (SDH) of all in cells indicate oxygen stress and energy crisis and mitochondria disturbances[9]. Our experimental results shows that 40 ppm Zn inhibit the carbohydrate synthesis from 39 mg/g to 29 mg/g in *Salvinia* and 18 mg/g to 13 mg/g in *Spirodela* from 4 to 12 days exposure duration respectively. The percent inhibition of carbohydrate during 12 days exposure is 50.85% in *Salvinia* and 64.87% in *Spirodela* in comparison to its respective control.

The 5 ppm of Zn in *Spirodela* promotes the protein synthesis and is directly proportional to exposure duration. The rate of increase is 6.8mg/g (9.67%), 7.1 mg/g (10.93%) and 48.4mg/g (21.73%) respectively at 4, 8 and 12 days exposure duration. In *Salvinia* also at 5 ppm of Zn promotes the protein synthesis by 47.8% (5.6mg/g), 45.58% (6.1mg/g) and 46.6% (6.6 mg/g) respectively at 4, 8 and 12 days exposure in comparison to control. The proteins are one of the most important group of biomolecules, includes maintenance of osmotic balance, storage of some particular elements, enzymes to catalyse biochemical reactions. Our investigation revealed the stimulation percentage of protein during 12 day exposure is 46.66 % in *Salvinia*, 21.73% in *Spirodela* compare to control. Similar observations were in *Potamogeton pectinatus* at lower concentration of Zn[27]. Phytochelatin (PC) produces enzymes like Glutathione reductase and PC synthetase bind and sequesters metal toxicity in the plant cell and increases the protein content at lower concentration[21,35].

However, the higher concentration of 40ppm in *Salvinia* was decreased by 2.57% (3.8mg/g), 19.05% (3.4mg/g) and 40.82% (2.9mg/g). Similarly in *Spirodela* at 40ppm of Zn, the percent inhibition was 43.54% (3.5mg/g), 53.12% (3.0mg/g) and 65.2% (2.4mg/g) at 4,8 and 12 days exposure in comparison to respective control (Fig. 1 and 2). Further 40ppm of Zn found to inhibit the protein synthesis from 3.8mg/g to 2.9mg/g in *Salvinia* and 3.5mg/g to 2.4mg/g in *Spirodela* from 4 and 12 days exposure respectively. The percent inhibition of protein is 40.82% in *Salvinia* and 65.82% in *Spirodela* during 12 days exposure duration, compared to control. The decline in protein content at high metal concentration may be due to the oxidation of protein[33] and also due to increased activate of protease or other catabolic enzymes which are activated and destroy the proteins. The high concentration of Zn metal ions binds with –SH functional groups resulting in the disruption of protein synthesis pathway.

Profile of Metal accumulation :

Fig. (3) shows the concentration of Zn accumulation in *Salvinia* and *Spirodela* was directly proportional to its concentration and exposure duration. The accumulation of metal in test plants at 4 days duration is more pronounced irrespective of exposure duration. However, at remaining duration of exposure it remains marginal.

The *Salvinia* exposed to 5ppm found to accumulate 2675.25 µg/g, 2998.25 µg/g and 3121.75 µg/g during 4, 8 and 12 days exposure duration respectively. However, at subsequent exposure duration, it shows marginal increase in their accumulation. *Salvinia* grown in experimental ponds containing 40ppm of Zn found to accumulate 17525.00 µg/g, 18130.75 µg/g and 18481.75 µg/g respectively at 4, 8 and 12 days exposure (Fig. 3). The *Spirodela,* test plant exposed to 5ppm of concentration accumulated 2802.0 µg/g, 3088.0 µg/g and 3403.0 µg/g respectively at 4, 8 and 12 days exposure. Similar to *Salvinia*, *Spirodela* at 40ppm concentration, Zn accumulation was 16925.0 µg/g. 17125.0 µg/g and 17200.0 µg/g respectively at 4, 8 and 12 days exposure. (Fig. 3).

Application of Two way ANOVA, it is found that both concentration and exposure duration are significant at $P < 0.01$ level in *Salvinia*. Further Dunet's test is applied for multiple comparison between control and different concentration, it is clear that all treatment means significantly differ with control. However, in case of *Spirodela*, by applying Two way ANOVA it is found that both concentration and exposure duration are not statistically significant (Table-5).

The Zinc is an essential micronutrient required for several metabolic activities of the plants and has a long biological half life[19]. Heavy metal pollution of water is a major environmental concern, is increasing at alarming rate due to anthropogenic activities. In the present investigation aquatic macrophytes viz, *Salvinia* and *Spirodela* are used to measure accumulation status.

It was found that *Spirodela* to accumulated maximum content of Zn (3403.0 µg/g) from the experimental pond containing 5.0 ppm zinc followed by *Salvinia* (3121.75 µg/g) during an exposure duration of 12 days. The accumulation of Zn in the plants exposed to 40ppm are as follows: *Salvinia* (1848.75 µg/g) following *Spirodela* (17200 µg/g) during 12 days exposure. The accumulation of Zn in plants exposed to 40 ppm of Zn are as : *Salvinia* (18481.75 µg/g) followed by *Spirodela* (17200 µg/g) during 12 days exposure. Our findings confirms observation of Jain and they found that Pb anf Zn content in *Azolla pinnata* and *Lemna minor* increased at initial concentration[25]. The rate of accumulation of Zn in *Potamogeton pectinatus* increases with increase in the exposure duration[37]. There are two phases in metal absorption by the plants. The first phase is rapid while 2nd phase is slow and extended. Our results are in total agreement with the above statement. The increase in the accumulation might be due to increased number of binding sites for the complexation of heavy metal ions, leading to the increased absorption, however, slow accumulation may be attributed to binding ions to the plants and establishment of equilibrium status between adsorbant and adsorbate.

It is concluded from the findings that the morphological, biochemical responses and metal accumulation profile by *Salvinia* and *Spirodela* were directly proportional to concentration of metal and maximum metal uptake was recorded at 4 days exposure and later it was marginal at subsequent concentrations and exposure duration, *Spirodela polyrhiza* is found to be suitable candidate for toxicity evaluation. However, the *Salvinia molesta* is the tolerant species and can be used for remediation of heavy metals from the aquatic ecosystem and environmental monitoring.

The authors are thankful to the Principal, B.L.D.E.A's Degree College, Jamkhandi (587301) India, Basaveshwar Science College Bagalkot, Shri Jagadambha Degree College Hittinahalli (L.T). and also JSS College Dharwad for providing necessary facilities to carry out research work.

References :

1. Allen, S.E., H.M. Grimshaw, J.A. Parkinson and C. Quarmby (1974) Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.
2. Arnon, D.I. (1949) *Plant Physiol, 24: 1-15.*
3. Azharbaig, M.B., K. Joseph, K. Vijay Rao and Jayantha (1999) *J. Ecotoxicol. Environ.*, *1:* 156-161.
4. Brix, H. and H.H. Schierup (1989) Ambio, *18:* 100-107.
5. Choudhary, M.P. and (2005) *Journal of Environmental Biology.*, *26*(1): 129-134.
6. Dammal, D., W.B. Green, M.T. Henzl, J.M. Hosea, R.H. Mc Pherson, J. Sneddon and M.D. Alexander, (1986) Recovery of heavy metals by immobilized algae. In: Thompson R (ed.) Trace metal removal from Aqueous solution, Whitstable Litho Ltd. . 1-24.
7. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, (1956) *Annul. Chem.*, *28:* 350-356.
8. Dushenkov, V., P.B.A.N. Kumar, H. Motto and I. Raskin, (1995) *Environ. Sci. Tech.*, *29:* 1239-1245.
9. Halliwell, B. and J.M.S. Gutteridge, (1993) Free radicals in biology and medicine. Clarendan Press Oxford.
10. Jain, S.K., P. Vasudevan, and N.K. Jha, (1990) *Water Res., 24*(2): 177-183. 1990.
11. Kuyack, N. and B. Voleksy, (1988) *Biotech Letto. 10:* 137-142.
12. Lowry, O. H., N. J. Rosebrough, R. J. Randall and A. Farr (1951) *J. Biol. Chem.*, *193:* 265-275.
13. Mathad, P., S.B. Angadi and R.D. Mathad (2004) *Asain Jr. of Microbiol. Biotech. Env. Sci.*, *6*(1): 99-106.
14. Mudhakavi, J.P. and B.V. Narayana (1997) *IJEP.*, *18*(2): 108. 1997.
15. Pahlasson, A.M.B. (1989) *Water, Air and Soil Pollut.*, *47:* 278-319.
16. Pandey, S., R.K. Asthana, A.M. Kayastha, N. Singh, and S. P. Singh, (1991) *Plant Physiol.*, *154:* 634-640.
17. Prasad, K.V.S.K., P. Pradha Sardhi, and P. Sharmila, (1999) *Environ. Expt. Bot.*, *42 :* 1-10. 1999.
18. Prasad, M.N.V. Trace metal In: *Plant ecophyscology* (Ed. Prasad, M.N.V.) John Wiley and Son. New York. 1997. 207-249.
19. Rai, A.K. and S. Kumar, (1999) *Applied Microbiol. Biotechno.*, *39:* 661-667.
20. Rajendra, J., Muthukrishnan and P. Gunashekaran, (2003) *Indian Journal of Experimental Biology.*, *41:* 935-944.
21. Romero- Puertas, M.C., F.J. Corpus, M. Rodriguez-Seranno, M. Gornez, and L.A. Dei Rio, (2007) *J. Plant Physiol.*, *164:* 1346-1357.

22. Satyakala, G. and K. Jamil, (1992) *Bull. Environ. Contam. Toxicol.*, *48:* 921-928.
23. Saygideger, S. and M. Dogan, (2009) *Bull. Environ. Contam. Toxicol.*, *73:* 182-189.
24. Sharma, R. K. and M. Agrawal (2005) *J. Environ. Biol.*, (**2** supl) : 301-313.
25. Sibihi, K., O. Cherifi, A. Agarwal, B. Oudra, and F. Aziz, (2012) *J. Mater Environ. Sci*., *3:* 497-506.
26. Singh, N.K., G.C. Pandey, U.N. Rai, R.D. Tripathi, H.B. Singh, and D. K. Gupta, (2005) *Bull. Environ. Contam. Toxicol.*, *74:* 857-863. 2005.
27. Sinha, S., M. Gupta, and P. Chandra, (1994) *Indian J. Envi. Hlth.*, *38:* 51-53.
28. Sinha, S. and K. Pandey, (2003) *Bull. Environ. Contam. Toxicol*., *71:* 1175-1183. 2003.
29. Sobero, M.C., J. Beltarano, and A. E. Ronco, (2004) *Bull. Environ. Contam. Toxicol*., *73:* 416-423.
30. Tripathi, R.D., U.N. Rai, P. Vajpayee, M.B. Ali, E. Khan, D.K. Gupta, M.K. Shukla, S. Mishra, and S. N. Singh, (2003) *Bull. Environ. Contam. Toxicol.*, *71:* 255-262.
31. Vajapayee, P., U.N. Rai, M.B. Ali, R. D. Triphati, V. Yadav, S. Sinha and S.N. Singh (2001) *Bull. Environ. Contam. Toxicol*., *67:* 246-254.
32. Van Assche, F. and H. Clijsters (1990) *Plant. Cell Environ.*, *13:* 195-206.
33. Wang. T.C., J.C. Weissman, G. Ramesh, R. Varadarajan and J.R. Bennemann (1996) *Bull. Environ.Contam. Toxicol. 57:* 779-786.
34. Wilde, E. W. and J. R. Benemann (1993) *Biotech. Adv.*, *11:* 781-812.
35. Wintz, H., T Fox, and C. Vulpe, (2002) *Biochem Soc. Transactions, 30:* 766-768.
36. Yongpisanphop, J., M. K. Chue, and P. Porethitiyook, (2005) *Journal of Environmental Biology., 26*(1): 79-89.

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 11, Issue, 04 (C), pp. 38144-38150, April, 2020

ISSN: 0976-3031

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

HEAVY METAL ACCUMUATION IN VEGETABLES CULTIVATED IN AGRICULTURAL SOIL IRRIGATED WITH SEWAGE AND ITS IMPACT ON HEALTH

*[1]N.M, Rolli., [2]Gurumath, S.V., [1]Chavan, R.R., [1]Anantpur, A .S and [3]Taranath, T.C

[1]BLDEA's Degree College Jamkhandi (587301), Karnataka, India
[2]M.G.V.C Arts, Commerce and Science College, Muddebihal, 586212, Karnataka, India
[3]Karnatak University Dharwad, Karnataka, India

DOI: http://dx.doi.org/10.24327/ijrsr.2020.1104.5249

ARTICLE INFO

Article History:

Received 6th January, 2020
Received in revised form 15th February, 2020
Accepted 12th March, 2020
Published online 28th April, 2020

Key Words:

Sewage, Toxicity, Xenobiotics, Food chain, Health, Accumulation

ABSTRACT

Unscientific management practices of pollutants lead to ecological imbalance. The use of sewage for irrigation is a common practice in majority of peri-urbans. The present study was focused to assess the levels of heavy metals *viz*, Pb, Cd, Mn, Ni, Zn and Cu in vegetables irrigated with sewage. The results represents sustainable build up of heavy metals in different parts of vegetables irrigated with sewage. The maximum accumulation of Zinc was noticed in Beetroot (72.9 ± 1.58 µg/g), copper in beetroot (5.90 ± 0.61 µg /g), Manganese in beetroot (171.14 ± 1.54 µg/g), Nickel in beetroot (5.90 ± 0.51 µg /g), lead in beetroot (30.40± 0.6 µg /g) and Cadmium in beetroot (2.91 ± 1.84 µg /g). The present study highlights that adults and children consuming vegetables grown in waste water irrigated soils ingest significant amount of these metals. However, the values of these metals were below the recommended maximum tolerable levels proposed by the WHO, expert committee on Food Additives (1999) and Kabata-Pendias, (2005). However, the regular monitoring of levels of these metals from sewage, in vegetables and in other food materials is essential to prevent excessive build up of these xenobiotics in the food chain.

INTRODUCTION

The rapid development of urbanization and industrialization, together with the shortage of availability of fresh water to be used for irrigation has led to raising use of sewage for agricultural land irrigation, mainly in periurban ecosystem due to easy availability and disposal problem. Irrigation with waste water is known to contribute significantly to the heavy metals content of the soil (Arora *et al*., 2008, Singh *et al:* 2010). Heavy metals are very harmful because of their non-biodegradable nature, long biological half lives and their potential to accumulate in different parts.

Most of the heavy metals are extremely toxic because of their solubility in water. Even low concentrations of heavy metals have damaging effects to man and animals because there is no good mechanism for their elimination from the body (Ashish Sharma *et al*., 2016). Excessive accumulation of heavy metals in agricultural soil through waste water irrigation, may not result in soil contamination, but also affect food quality and safety (Chen Wang and Wang, 2005). The use of sewage has significant health implications for both farmers and consumers. Therefore, for better satisfactory result, waste water should be treated to remove harmful substances and microorganisms before used is for irrigation (Phytoremediation technique). Special attention has been paid to those vegetables that are eaten raw. Since the microorganisms that settle over them are able to survive for several weeks and when these vegetables are consumed, they produced diarrhea, salmonellosis, shigellosis (Zhaung P *et.al;* 2009) The indiscriminate use of causes clogging of soil pores resulting in decreased permeability. Lack of aeration in sewage produces toxic gases which were found to create unhygienic conditions (Guerra *et al;* 2012).

Further more, the consumption of heavy metal contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behavior, disabilities associated with malnutrition and a high prevalence of upper gastro-intestinal cancer *(Orisakwe et al;* 2012; Alam *et al.*, 2003; Dube *et al.*, 2004).The present study was conducted with an aim to compare the heavy metals accumulation potential in different parts of vegetables. The effect of irrigation with waste water is also studied in different vegetables to which human beings are exposed and it is found

Corresponding author: **N.M, Rolli**
BLDEA's Degree College Jamkhandi (587301), Karnataka, India

at proper health education is indispensible in relation onsumption of vegetables.

MATERIALS AND METHODS

The experiments were conducted at Environmental Science Laboratory, Karnatak University Dharwad and BLDEA's Environmental Science Laboratory, Degree College, Jamkhandi. Samples of some commonly grown vegetables viz, beetroot (*Beta vulgaris*), Tomato (*Solanum lycopersicum*), Pudina (*Mentha spicata*), Brinjal (*Solanum melongena*), Coriander (*Coriandrum sativum*) and Carrot (*Daucas carota*) were collected from Madihal (Periurban area nearer to Dharwad city) which is one km away from the main sewage canal from the field which is irrigated with sewage, however, Ammanagatti village is selected as a control site, where the farmers were using bore well water for the irrigation (Fig.3).

The vegetables were collected very randomly from three different sites (results were in triplicates). Experimental samples were collected in a large presterilized containers and transported to the laboratory for various parameters of sewage, control and vegetables. Sewage and control (bore well water) were analyzed for its physico chemical parameters as per standards methods (APHA- 1998) and Na(Sodium) is analysed using Atomic Absorption Spectrophotometer. Soil samples, vegetable samples of sewage and bore well were analysed for heavy metals present using mixed acid digestion method (Allen *et al.,* 1974) soil samples irrigated with sewage and bore well were collected with an average depth of 5.20cm randomly where vegetables were growing.

The soil is air dried, sieved to desired particle size for analysis. Similarly for the estimation of heavy metals in vegetables were dried at 80^0 C in an oven for 24 hours to get constant weight. The dried part (Root, Stem, Leaf and Fruit) of the plant individually is homogenized with a blender to a powdery form. One gram of each sample was separately digested by using analytical grade (AR) chemicals such as nitric acid (HNO_3), Sulphuric acid (H_2SO_4) hydrogen peroxide (H_2O_2) and perchloric acid (60%) in Gerhardt digestion unit.

The solution was filtered using whattman filter paper number, 44 in a volumetric flask by adding distilled water and final volume is made to 100 ml and analyzed for heavy metals such as zinc (Zn, wavelength 213.9 nm), copper (Cu, wavelength 324.7 nm), Manganese (Mn, wavelength 279.5 nm), Nickel (Ni, wavelength 232.0 nm), , Lead (Pb, wavelength 217.0 nm) and Cadmium (Cd, wavelength 217.0 nm) with GBC -932 plus Atomic Absorption Spectrophotometer (Australia) with an air / acetylene) flame and their respective wavelength metal hollow cathode lamps. Standard solution for heavy metals were purchased from Siscochemical Laboratory Bombay (1000 mg/lt). The working standards were prepared by serial dilutions of standard stock solutions and were used for the calibration of the instrument. The experiment results were in triplicate.

Table 1 Comparison of sewage with WHO standards

Parameters	pH	Odour	EC	TDS	Hardness	Alkalinity	COD	Chloride	Ca	Mg	Na	SO_4
Sewage	7.3	Foul smell	1.193	750	350	424.5	241	421	81	74	121	52
WHO (1993)	6.5 – 8.8	Acceptable	1400 µohms	1000	500	120	5	250	100	150	200	250

Note : All parameters are in mg/lt except pH and EC

Table 2 WHO Standards

Heavy metals	Plants	Water
Zinc	50	5.0
Copper	10	2.0
Manganese	--	--
Nickel	10	0.2
Lead	2	0.05
Cadmium	10	2.0
Iron	20	1.0

Plant - µg/g, water - µg/ml

Table 3 Kabata- Pendias (2005)

Heavy metal	Sufficient or normal	Excessive or toxic	Tolerable in agricultural crops
Zinc	27-150	100 – 400	300
Copper	5 – 30	20 – 100	50
Manganese	30 – 300	400 – 1000	300
Nickel	0.1 – 5	10 -100	50
Lead	5 – 15	30 – 300	10
Cadmium	0.05 – 0.2	5 – 30	3
Iron	375 – 400	600 – 700	500

Table 4 Vegetable edible part used

Common name	Scientific name	Family	Part used
Beet root	*Beta vulgaris* L.	Amaranthaceae	Root
Coriander	*Coriandrum sativum* L.	Apiaceae	Leaf
Tomato	*Solanum lycopersicum* (L) H.	Solanaceae	Fruit
Carrot	*Daucas carota* L	Apiaceae	Root
Pudina	*Menthe spicata* L.	Lamiaceae	Leaf
Brinjal	*Solanum melongena* L	Solanaceae	Fruit

RESULTS AND DISCUSSION

The physicochemical characteristics of sewage sampled from the experimental site were presented in the table. 1. The sewage was dark brown in colour with unpleasant odour. All the physicochemical parameters were within WHO (1993) range except chloride, COD and alkalinity. The alkalinity of the sewage is due to soap and detergents (WHO- 1993) (Table 1).

Sewage also contains excess content of heavy metals. The heavy metals except Zn, Cu, Mn exceeding the sufficient and normal status but are tolerable level in agricultural crops (Kabata-Pendias, 2005). The concentration of heavy metals in different vegetables with respect to different parts are presented in the table 5 and 6. The data analysis represents the metal translocation and accumulation potentiality vary from vegetable and also different parts of the vegetables also. Among the vegetables the underground edible parts and fruit bearing vegetables shows significant accumulation of metals. The heavy metal concentration in edible parts of vegetables are shown in table 4,5 and 6, and in fig. 1 and 2. It was clearly observed that the concentration of all heavy metals is higher in sewage irrigated vegetables than the bore well water (control) irrigated plants. The above figs. and tables represents high concentration of heavy metals in vegetables irrigated with sewage.

Table 5 Accumulation profile of heavy metals in vegetables

Vegetables		Zn		Cu		Mn	
		Control	Sewage	Control	Sewage	Control	Sewage
Beetroot	Root	21.10±1.20	72.9±1.58	4.09±0.30	5.90±0.61	25.41±1.20	171.14±1.54
	Stem	19.08±1.94	65.8±1.45	4.01±0.85	4.4±0.35	21.44±1.26	164.18±1.84
	Leaf	18.01±0.68	61.1±1.41	3.01±1.58	4.2±1.49	19.51±1.40	151.14±0.72
Tomato	Root	15.31±1.68	49.8±1.45	4.01±1.13	5.80±1.81	22.11±1.30	121.14±1.25
	Stem	14.01±1.13	40.9±1.20	3.01±1.41	5.15±1.42	20.41±1.55	54.80±1.30
	Leaf	13.41±1.02	34.7±1.13	3.04±1.47	4.92±1.05	19.80±0.72	51.74±1.85
	Fruit	12.91±1.51	31.4±1.02	3.03±1.81	4.81±1.41	19.75±0.77	48.82±1.30
Pudina	Root	20.91±0.98	41.8±0.72	4.10±1.47	5.70±1.05	26.11±1.52	118.4±0.51
	Stem	18.04±1.12	40.4±1.45	4.08±1.81	5.61±1.41	21.80±0.30	112.4±0.91
	Leaf	17.04±1.51	38.18±1.69	3.91±1.13	4.91±1.81	18.14±1.72	112.4±1.40
Brinjal	Root	15.01±1.64	44.8±1.45	4.10±1.41	5.91±0.61	22.19±1.50	123.14±1.5
	Stem	13.94±1.13	38.4±1.20	3.41±1.84	5.84±1.49	20.41±0.60	56.80±0.60
	Leaf	13.11±1.02	34.5±1.13	3.42±1.84	5.41±1.42	19.5±1.05	55.74±1:14
	Fruit	10.12±1.51	31.8±1.09	3.41±1.81	5.42±1.05	10.3±0.77	49.82±0.72
Coriander	Root	14.91±1.49	42.8±0.72	4.11±1.47	5.64±1.81	26.01±0.30	117.41±0.61
	Stem	13.11±1.04	40.9±1.45	4.09±1.81	5.10±1.47	20.91±0.85	113.54±0.35
	Leaf	13.01±1.68	38.41±1.2	3.91±1.84	4.92±1.81	17.40±1.18	109.51±1.49
Carrot	Root	20.10±1.20	71.8±1.38	4.14±0.30	5.94±0.35	25.91±1.87	168.41±1.25
	Stem	14.13±1.94	64.9±1.45	4.04±1.54	5.15±0.61	22.40±1.55	159.48±1.38
	Leaf	18.04±0.68	60.01±1.12	3.91±1.13	4.51±1.41	18.51±1.26	148.41±0.98
Soil		20.24±1.16	47.56±1.42	8.42±1.11	24.12±1.05	18.85±1.65	202.6±1.15
Water		4.15±1.17	24.03±1.62	31.3±1.22	191.02±0.84	241.26±2.31	310.3±1.58

Vegetables & Soil: µg/g
Sewage / fresh water: µg/lt

Table 6 Accumulation profile of heavy metals in vegetables

Vegetables		Ni		Pb		Cd	
		Control	Sewage	Control	Sewage	Control	Sewage
Beetroot	Root	3.06±1.50	5.91±0.51	9.12±1.52	30.40±0.60	1.60±0.60	2.91±1.84
	Stem	2.91±0.60	5.01±0.92	8.09±0.83	25.21±1.14	1.22±1.42	2.82±0.92
	Leaf	1.92±1.05	4.41±1.40	7.05±0.81	21.18±1.12	1.12±0.64	2.76±1.52
Tomato	Root	1.91±1.87	5.01±1.40	5.01±2.51	22.10±1.12	1.08±1.14	2.76±1.14
	Stem	1.25±1.55	4.98±1.71	4.92±2.62	19.42±1.14	1.06±1.10	2.75±0.72
	Leaf	1.16±1.42	4.51±1.25	4.40±1.82	18.01±0.72	1.04±1.12	2.45±0.64
	Fruit	1.04±1.25	4.41±1.05	3.94±2.42	7.12±0.64	1.00±0.60	2.41±0.86
Pudina	Root	1.81±1.50	5.09±1.14	5.02±1.40	29.42±0.86	1.60±1.40	2.72±0.72
	Stem	1.50±0.60	4.58±0.64	4.91±0.92	22.14±0.82	1.42±1.10	2:64±0.64
	Leaf	1.14±1.05	4.12±0.72	4.60±0.60	16.41±0.78	1.21±1.81	2.42±0.92
Brinjal	Root	1.89±1.25	5.02±1.21	5.04±0.80	28.92±0.80	1.08±0.60	2.78±0.61
	Stem	1.21±1.22	4.81±1.25	4.82±0.48	24.14±0.42	1.04±0.30	2.64±0.81
	Leaf	1.15±1.52	4.48±1.52	4.38±0.64	18.22±0.62	1.01±0.80	2.52±1.24
	Fruit	1.04±1.34	4.21±1.36	3.84±0.82	18.08±0.92	0.92±0.60	2.40±0.64
Coriander	Root	1.81±1.52	5.02±1.40	5.22±1.34	26.82±0.82	1.62±0.86	2.42±1.42
	Stem	1.49±1.23	4.58±1.36	4.92±1.12	24.82±1.68	1.58±0.64	2.40±0.60
	Leaf	1.11±1.25	4.14±1.52	4.62±1.68	18.18±1.38	1.32±1.52	2.38±T.10
Carrot	Root	3.08±0.96	5.8±1.68	8.98±1.12	28.92±0.61	1.80±1.62	3.01±0.64
	Stem	2.90±1.58	5.01±1.75	8.04±1.34	24.82±0.82	1.62±1.10	2.91±1.42
	Leaf	1.80±1.26	4.42±0.95	6.92±0.64	20.42±0.68	1.54±1.12	2.75±0.82
Soil		1.81±0.51	8.86±1.13	8.12±1.45	34.01±0.94	1.64±0.41	3.41±0.84
Water		59.5±1.70	58.97±1.57	18.41±1.04	54.91±1.15	2.04±0.51	8.42±0.41

Vegetables & Soil: µg/g
Sewage / fresh water: µg/lt

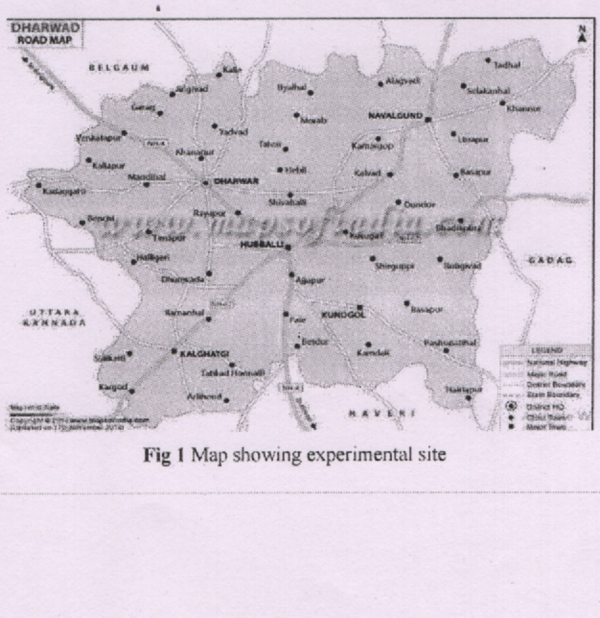
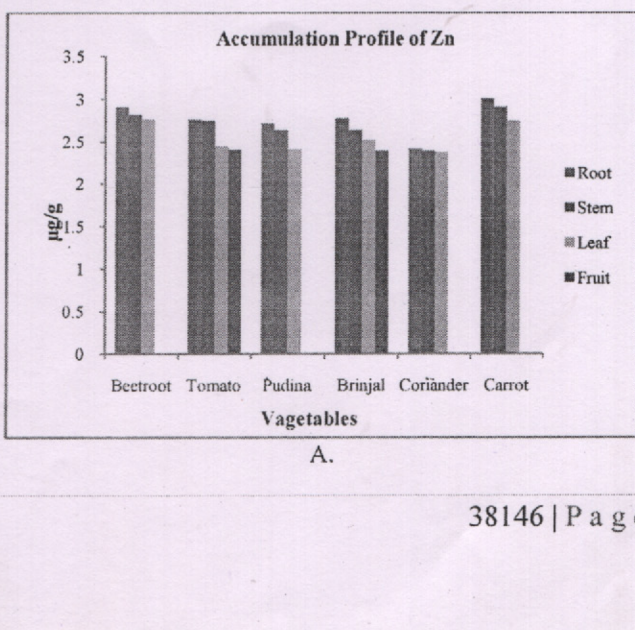
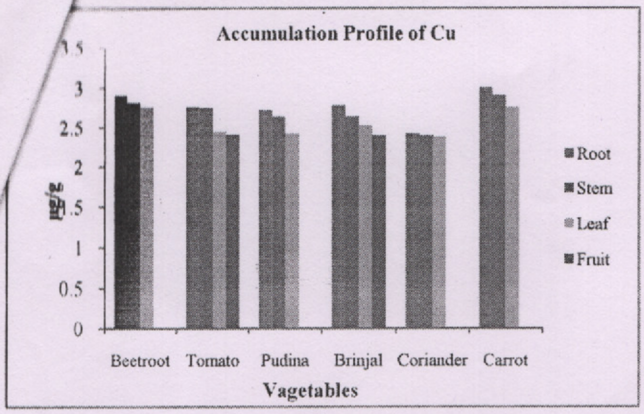


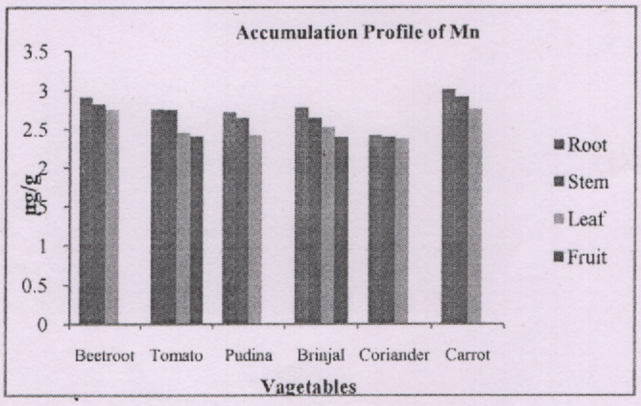
Fig 1 Map showing experimental site



A.



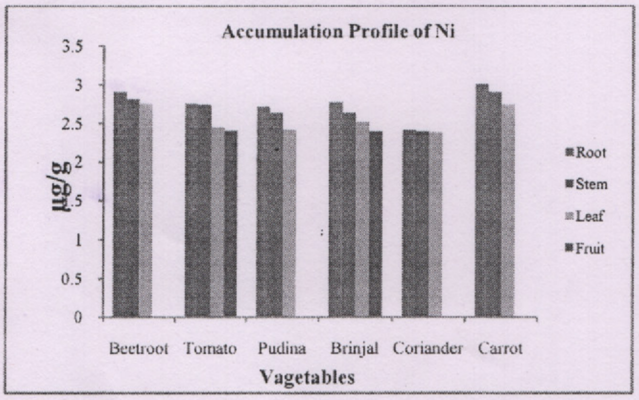
B



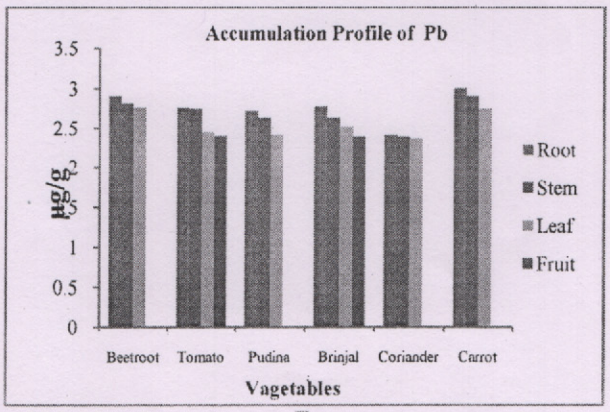
C

Fig 1 Metal accumulation profile in vegetables

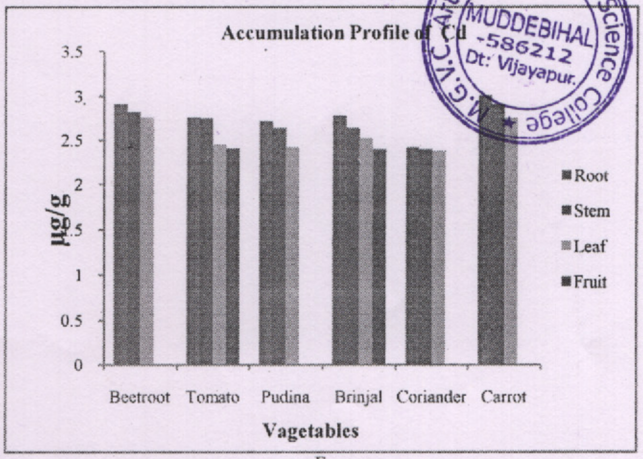
A. Zinc B. Copper C. Manganese



D



E



F

Fig. 2. Metal accumulation profile in vegetables

D. Nickel E. Lead F. Cadmium

Zinc

The tolerable limit of agricultural crops for zinc is 300 µg/g and normal is 27-150 µg/g. Among the vegetables the underground edible parts shows significant accumulation of metals compared to other parts of vegetables.

Beetroot (root) > Carrot (root) > Coriander (leaf) > Pudina (leaf) > Brinjal (fruit) > Tomato (fruit)
72.9±1.58 µg/g > 71.8±1.38 µg/g > 38.41 ± 1.2 µg/g > 38.18 ±1.69 µg/g > 31.8 ± 1.09 µg/g > 31.4 ± 1.02 µg/g
In the vegetables irrigated with fresh water recorded maximum in (18.04 ± 0.68 µg/g) and minimum was recorded in brinjal fruit (10.12 ± 1.51 µg/g). However, sewage irrigated beetroot was found to accumulate to the extent of 72.9±1.58 µg/g and minimum was recorded in Pudina leaf to the extent of 38.18 ± 1.69 µg/g. Zinc is considered to be relatively nontoxic, however, excess amount can cause system dysfunctions that result in the impairment of growth and reproduction. The clinical signs of zinc toxicosis have been reported as vomiting, diarrhoea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anaemia (Duruibe *et al.,* 2007), Zinc is nutritionally essential metal but excess in the body bound in various transcription regions such as polymerase enzymes (Wang *et al.,* 1997).

Copper

Tolerable limit in agricultural crops for Cu is 50 µg/g and normal limit is about 5-30 µg/g. The vegetables irrigated with fresh water contains maximum 4.14 ± 0.30 µg/g in carrot root and minimum in beet root leaf to the extent of 3.01±1.58 µg/g. However, maximum accumulation of Cu was recorded with irrigated (sewage) crop carrot (root) to the extent of 5.94 ± 0.35 µg/g and minimum was recorded in 4.21 ± 1.49 µg/g in beet root leaf. Copper is essential element, but higher concentration intake leads to severe mucosal irritation, widespread capillary damage (Salmeron and Pozo, 1989). Excessive accumulation of copper in liver, brain, kidneys, menifests into 'Wilson disease' (This disorder is also called hepatolenticular degeneration).

Carrot (root) > Beetroor (root) > Brinjal (fruit) > Coriander (leaf) > Pudina (leaf) > Tomato

0.35 μg/g > 5.9± 0.61μg/g > 5.42± 1.05μg/g > 1.81 μg/g > 4.91± 1.81 μg/g > 4.81 ± 1.41 μg/g

...nganese

...lerable limit for Mn in agricultural crops is 300 μg/g and ...ormal value is 30-300 μg/g (Kabata- Pendias, 2005). In our observation maximum content of Mn was recorded in Pudina leaf- 26.11 ± 1.52 μg/g and minimum content in coriander leaf– 17.40 ± 1.18 μg/g, irrigated with fresh water. However, the order of accumulation in different vegetables irrigated with sewage is:

Beetroot (root) > Carrot (root) > Pudina (leaf) > Coriander (leaf) > Brinjal (fruit)

> Tomato
171.14±1.54μg/g > 168.41±1.25μg/g > 112.41±1.40μg/g > 109.51±1.49 μg/g > 49.82± 0.72μg/g > 48.82 ± 1.30 μg/g

Manganese is micronutrient, essential for physiological functions. At higher concentrations it is toxic causing neuropsychiatic disorder characterized by irritability, difficulty in walking and speech disturbances (Singh and Kalamdhad, 2011).

Nickel

Nickel has been considered to be an essential trace element for human health (animal) (Zighan et al., 2012). The permissible tolerable limit in agricultural crops (Kabata-Pendias, 2005) is 50 μg/g and normal is about 0.1 to 5.0 μg/g. In carrot root Ni was found to accumulate to the extent of 3.08 ± 0.96 μg/g in carrot root and minimum accumulation was recorded in 1.04± 1.25 μg/g in tomato fruit, when the vegetables were treated with bore well water (control). However, the vegetables treated with sewage the accumulation leads to the extent of 5.91± 0.51 μg/g in beet root (root) and minimum record was noticed in pudina leaf 4.12 ± 0.72 μg/g.

Due to bioaccumulation Ni shows skin irritation, damage to the lungs, nervous system and mucosa membranes. When present easily distributed to kidneys, pituitary, lungs, skin, adrinals, ovaries and testis but however, risks were highest for lungs and nasal cancers (Singh and Kalamdhad, 2011). However, the order of accumulation in different vegetables irrigated with sewage is as follows:

Beet root (root) > Carrot (root) > Tomato (fruit) > Brinjal (fruit) > Coriander(leaf) > Pudina (leaf) 5.91± 0.51μg/g > 5.8 ± 1.64μg/g > 4.41 ± 1.05μg/g > 4.21 ± 1.36μg/g > 4.14 ±1.52μg/g > 4.12± 0.72 μg/g

Nickel occurs mainly in the form of sulphide and silicate minerals. Nickel when administrated to animals is rapidly distributed to kidneys, pituitary, lungs, skin, adrenals, ovaries and testis (Sunderman, 1989). Nickel is carcinogenic to human and risks are highest for lung and nasal cancers. Nickel also damages DNA directly through reactive oxygen species (McCoy and Kinney, 1992).

Lead

The permissible tolerable limit in agricultural crops is 10.0 μg/g and normal is varying from 5.0 to 15.0 μg/g (Kabata-Pendias, 2005). The vegetables grown using bore well water contains maximum in carrot (root) to the extent of 9.12 ± 1.52 μg/g and minimum was noticed in brinjal fruit to about 3.84 ± 2.41 μg/g. However, the vegetables grown in using sewage contain maximum in beet root (root) to about 30.40 ± 0.60 μg/g and minimum in tomato fruit to the extent of 7.12 ± 0.64 μg/g.

Lead is a poisonous metal, in human it is directly absorbed into the blood stream and is stored in soft tissues, bones and teeth. Due to bioaccumulation it leads to chronic damage to Central Nervous System (CNS) and Peripheral Nervous System (PNS). Lead can also causes difficulties in pregnancy (Peokjoo et al., 2008). Lead is ubiquitous metal and predominantly poisoning children. Children absorbs greater proportion of lead than adults and also it induce aberrant gene transcription (Boutan *et al.*, 2001). Lead brings weakness, anemia, kidney and brain damage and even death. It has been reported that lead can cross the placental barrier, implying that pregnancy women may results in defects to the unborn foetus. However, the order of accumulation of Pb in different vegetables irrigated with sewage is as follows:

Beetroot (root) > Carrot (root) > Coriander (leaf) > Brinjal (fruit) > Pudina (leaf) > Tomato (fruit) 30.40 ± 0.6 μg/g > 28.92 ± 0.61μg/g > 18.18±1.38 μg/g > 18.08 ±0.92 μg/g > 16.41±0.78 μg/g > 7.12 ± 0.64 μg/g

Cadmium

The permissible tolerable limit in agricultural crops for Cd is 3.0 μg/g and normal is varying from 0.05 to 0.2 μg/g (Kabata-Pendias – 2005). In control experimental vegetables (fresh water) maximum accumulation is recorded in carrot (root) is 1.80 ± 1.62 μg/g and minimum was recorded in brinjal fruit to 0.92 ± 0.60 μg/g. However, sewage irrigated vegetables shows surprising results and maximum accumulation was noticed in carrot (root) is 3.01 ± 0.64 μg/g and minimum was recorded in 2.38 ± 1.10 μg/g (coriander leaf). However, the order of accumulation of cadmium in different vegetables irrigated with sewage is as follows:

Carrot (root) > Beetroot (root) > Pudina (leaf) > Brinjal (fruit) > Tomato (fruit) > Coriander (leaf) 3.01 ± 0.64μg/g > 2.91±1.84 μg/g > 2.42 ± 0.92μg/g > 2.40 ± 0.64 μg/g > 2.41 ± 0.86 μg/g > 2.38 ± 1.10 μg/g

Cadmium accumulate in the human kidney, respiratory system, cardiac failure and is also associated with bone diseases (Singh and Kalamdhad, 2011). Cadmium affects the transcription of number of genes (Schmidt, 1995). Wang and Crowley (2005) also reported that discruption in the transcription of genes in coding ribosomal proteins explains molecular mechanism of cadmium toxicity. International Agency for Research on Cancer (IARC) has introduced Cd as a Carcinogenic agent which is a main cause of kidney dysfunction.

It has been reported that the entry of heavy metals to the food chain through vegetables consumption (Dubey *et al.*, 2004: Liu *et al.*, 2006). The usage of sewage, contaminated vegetables with heavy metals has significant health implications for both farmers and consumers (Birley *et al.*, 2002; Alam *et al.*, 2003). Lead cumulative poisoning effects are with haematological damage,. anaemia, kidney malfunctioning, brain damage etc (Patra *et al.*, 2001). Cadmium is also one of the serious environmental pollutants. Cadmium enters the organism primarily via the alimentary and ratory tract. Cadmium damages specific structure of the ctional unit of kidney (Lal, 2005; Tuzen *et al.*, 2005) and eletal deformities commonly known as itai – itai disease Anonymous, 1997). Thus, the increased circulation of heavy metals in vegetables results in inevitable build up of toxins in the human food chain and their accumulation leads to various ailments. Our results shows the agreement with previous showing elevated levels of heavy metals in the edible part of the vegetables (Khan *et al.*, 2007).

The results of the present study reports that the vegetables grown using sewage constitute risk due to accumulation of metals. In our collected experimental samples it was recorded that heavy metal concentrations are above the permissible limit of WHO but are in the range proposed by Kabata – Pendias (2005) except Ni, Cd and Pb. It was agreed that the use of vegetables have potentiality to remove the metals from the soil, thereby, cleaning the environment, but it enters the food chain due to consumption. The sewage has significant health implications for both consumers and farmers, and hence, proper health education is quite essential for women and men who were in contact with sewage during farming (Hunashyal *et al.*, 2005).

Acknowledgement

The authors are thankful to the Principal, BLDEA's Com. BHS Arts & TGP Science College, Jamkhandi (Karnataka), Principal, MVC Degree College Muddebihal and Chairman, PG Department Botany, Karnatak University, Dharwad (Karnataka) for providing necessary facilities to carry out research work.

References

Agency for Toxic Substances and Diseases Registrary (ATSDR). 2005. Toxicological Profile for Nickel.Atlanta,GA: U.S. Department of Health &Human Services,Public Health Service,.

Alam, M.G.M., Snow, E.T. and Tanaka, A. 2003. Arsenic and heavy metal contamination of vegetables grown in Santa village, Bangladesh, *Science of the total Environment*. 308:83-96.

Allen, S. E., Grimshaw, H. M., Parkinson, J. A. and Quarmby, C. 1974. Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.

Anonymous. 1997. Report on Cd in fresh water fish. Water quality criteria for European fresh water fish. European Inland Fisheries Advisory Commission. F.A.O.U.N Technical paper No. 30.

APHA, AWWA, WPCF. 1998. Standard methods for the examination of water and waste water,

Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B., Mittal, N. 2008. Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chem*. 111:811-815.

Ashish Sharma, Jatinder Kaur and Avinash Kaur Nagpal. 2016. Heavy metals in vegetables: screening health risks involved in cultivation along waste water drain and irrigating with waste water. *Springerplus*. 5:488.

Aslam, M., Unlu, M. Y., Turkmen, N. and Yilmaz, Y. Z. (2003). Sorption of Cd and effects on growth, protein content and photosynthetic pigment composition of Nasartium officinal;e R. Br. And Mentha aquatic L. *Bull. Environ. Contam. Toxicol*. 71:323-329.

Birley, M. H. and Lock, K. 2002. "Health and periurban natural resource production" Environment and Urbanization. 10(1): 89-106.

Bouton, C.M. and Pevsner, J. 2000. Effects of lead on gene expression. *Neurotoxicology*., 21, 1045-1055.

Dube, B.K.., Sinha, P. and Chatarjee, C. 2004. Crop plants as biological tools for assessing and monitoring agricultural lands inundated with sewage and sludge, *Bull. Environ. Contam. Toxicol*. 72:429-436

Guerra, F., Trevizam, A.R., Muraoka, T., Marcante, N.C., Caniatti-Brazaca, S.G. 2012. Heavy metalks in vegetables and potential risk for human health. *Sci. Agric*. 69:54-60.

Hunshyal, C. S., Salakinkop, S. R. and Brook, R. M. 1997. Sewage irrigated vegetable production around Hubli-Dharwad, Karnataka, India. *The Kasetsarat Journal*. 32(5): 1-8.

Kabata Pendias, A. 2005. Heavy metals (problems and solutions), Edited by: W. Salomonas, U. Frostner and P. Mader), Springer Verlag, Berlin, Heidelberg, New York. London, Tokyo, 3-18.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z. and Zhu, Y.G. 2007. Health risks of heavy metals in contaminated soils and food crops irrigated with waste water in Veijing, Chaina. *Environment Pollution*, 1-7.

Lal, R.B. 2005. Effects of heavy metal toxicity and exposure on human health, Indian *J. Environ. And Ecoplan*. 10(2):533-536.

Liu, J., Xie, Y., Ward, J., Diwan, B.A. and Waalkies, M.P. 2006. Toxicogenomic analysis of aberrant gene expression in liver tumours and non-tumours livers of adult mice exposed in vitro to inorganic arsenic. *Toxicol. Sci*. 77:249-257

Mc Coy, H. and Kinney, M.A. 1992. A review of bio indications on Ni and Mg. It Immune system and oncology. *Magnes. Res*. 5: 223-232.

National Institute for Occupational Safety and Health (NIOSH). 1995. Report to congress on workers' Home contamination study conducted under the workers Family protection Act (29 U.S.C67/a) Publication No. 95-123.

Orisakwe, O.E., Niduka, J.K., Amadi, C.N., Dike and D.O. 2012. Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. *Chem Cent J*. 6:77-83.

Patra, U., Gupta, S., Talapatra, S.N., Giri, A.K. 2001. Genotoxic effects after invivo exposure of vegetables extracts containing heavy metals from Dhapa area of Calcutta, India, I effects of cauliflower, Spinach and radish, *Food and Chem, Toxicol*, 39:67-72.

Salmeron, J. and Pozo, R. 1989. Heavy metal uptake from greenhouse border soils for edible vegetables. *Journal of the Science of food and Agriculture*, 49: 307-314.

Schimidt, C.J. Jubier, M.F. and Hamer, D.H. 1995. Structure and expression of two human metallothionein-I isoform genes and a related pseudogene, *J. Biol. Chem*, 260: 7731-7737.

Seokjoo, Y., Sang, S.H. and Rana, S.V.S. 2008. Molecular markers of heavy metal toxicity- A new paradigm for health risk assessment. *Journal of Environmental Biology*, 29(1):1-14.

ingh, A., Sharma. R.K., Agarwal, M. and Marshall, F.M. 2010. Health risk assessment of heavy metals via dietary intake of foodstuffs from the waste water irrigated site of a dry tropical area of India. *Food Chem Toxicol*. 48:611-619.

Singh, J. and Kalamdhad, A.S. 2011. Effects of heavy metals on soil, plants human health and aquatic life. *International Journal of research in Chemistry and Environment*, vol. 1 issue 2, 15-21.

Sunderman, F.W. 1989. Jr: Mechanism of nickel carcinogenesis. Scand. *J. Work. Environ. Hlth*, 15: 1-12.

Tuzen, M., Parlor, K., Soylak, M. 2005. Enrichment / Seperation of Cadmium (II) and lead (II) in environmental samples by solid phase extraction. *J. Hazard Mat IN press*.

Wang, A. and Crowley, D.E. 2005. Global gene expression responses to cadmium toxicity in Escherichia coli. *J. Bacterol*, 187: 3259-3266.

Wang, W. and Lewis. M.A. 1997. Metal accumulation by aquatic macrophytes In: *Plants for environmental studies* (Eds: W. Wang, J.W. Gorsuch and J.S. Hugkes). CRC Press, New York. 367-416.

WHO. Evaluation of certain food additives &contaminants 41st Report of the Joint FAO/WHO Expert Committee on Food Additives.Tech 1993.Report Series,837.

World Health Organization. 1993. Guidelines for drinking water quality- I, Recommendations, 2nd Edi. Geneva WHO.

Zhuang, P., Zou, B., Li, N.Y., Li, Z.A. 2009. Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, Chaina: implication for human health, *Environ Geochem health*, 31:707-715.

How to cite this article:

N.M, Rolli *et al.*2020, Heavy Metal Accumuation in Vegetables Cultivated in Agricultural Soil Irrigated with Sewage and its Impact on Health. *Int J Recent Sci Res.* 11(04), pp. 38144-38150. DOI: http://dx.doi.org/10.24327/ijrsr.2020.1104.5249

****

PRINCIPAL,
M.G.V.C. Arts, Com. & Science College
MUDDEBIHAL - 586212.

Impact Factor 2017: 7.383

INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH

WWW.RECENTSCIENTIFIC.COM

ISSN:0976-3031

M.G.V.C. Arts, Commerce & Science College, MUDDEBIHAL - 586212 Dt: Vijayapur.

Certificate For Publication

Is hereby honoring this certificate to

N.M, Rolli., Gurumath, S.V., Chavan, R.R., Anantpur, A .S and Taranath, T.C

In recognition of the publication of the paper entitled

HEAVY METAL ACCUMUATION IN VEGETABLES CULTIVATED IN AGRICULTURAL SOIL IRRIGATED WITH SEWAGE AND ITS IMPACT ON HEALTH

Published in International Journal of Recent Scientific Research
(Vol. 11, Issue, 04 (C), pp. 38144-38150, April, 2020)

2020

INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH ★ IJRSR

ISSN 0976-3031

Signature:
Managing Editor

Date: 28/04/2020

Web: www.recentscientific.com
E-mail: recentscientific@gmail.com