

Using Photomicrograph for the Analysis of Chromosomes in Onion (*Allium cepa*), for Understanding the effect of the Lead (Lead Acetate) and Zinc (Zinc Sulphate) Salts on Chromosome by Sewage Water Irrigation



Biotechnology

KEYWORDS : Allium cepa, Zinc Sulphate, Lead Acetate, metacentric, submetacentric, subtelomeric

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ABSTRACT

*The aim of the present study was to investigate the genetic effects of some heavy metals on onion (*Allium cepa*) which is affects human beings directly. Since the beginning of the industrialization, heavy metal pollution of soil and aquatic ecosystems has been significantly increased. The primary reason for actuality of this problem in the human health risk resulting from intake of contaminants through drinking water or through the food chain. Onion is one of the major vegetable crops in India. So for the growing of onion water and soil is required. Untreated sewage water used in agriculture causes biomagnification of heavy metals and it has some genotoxic effects. Onions were obtained commercially and were placed on sand with the tested solutions of two heavy metals. For the control it was used distilled water. When root reached lengths of 2 to 3 cm, they were cut off and incubated with colchicines and fixed with fixative solution containing ethanol and acetic acid in a ratio 1:3. Again incubated with 1N HCl and washed with water. Then fixed roots were placed into staining solution and visualized. Photomicrographs were taken from the well spread preparations. All measurements were taken using the software images. In this study sample 1 has only one type of chromosome that is metacentric, sample 2 has all the three type of chromosome that are metacentric, submetacentric and subtelomeric where the control has two types of chromosome that are metacentric and submetacentric. There had been the change in total chromosome length (TCL), as well as the average chromosome length (ACL), The arm ratio, (p:q) was also affected by the Lead Acetate and Zinc Sulphate.*

INTRODUCTION:

As per the press release of the Govt. of India, the % of irrigated land is about 44%. India is still depend upon the monsoon rain for its agriculture. This agriculture accounts 80% of fresh water in India. This amount of consumption of water by agriculture is challenged by growing demands of the municipal & industrial need. Due to the climate change, population growth & urbanization demand in order to maintain the water supply to agriculture the sewage water is transfer to the agricultural land for irrigation purpose. The treated waste water volume in India is 11,787 million liter/day which irrigates about 70722 hectares of agriculture land and untreated waste water of about 26,467 million liter/day is used to irrigate 1032213 hectares of land.

The most notable & important in human life is water and it is diminishing and so the agricultural land. The onus is a scientist to find out this finite sources for reliable supply to the demand of the population what most scientists feel is that the using of domestic waste water for growing paddy, vegetables, fruits, feed stock etc. But this use of sewage water leads to contamination of soils as well as the ground water consists of various dangerous chemicals such as the pesticides, fungicides, toxic xenocompounds & heavy metals. Consumption of the agricultural products grown with the help of sewage water by humans for longer period can cause diseases & bio magnification of some of the heavy metals. The xeno compounds can change the micro flora of the soil, it can change soil characteristics, the pH of the soil, soil salts and soil electrical conductance etc. The sewage domestic water degrades the soil and affects the soil health. The sewage water also contains the microorganisms which is harmful for human health because of the pathogenic microorganism will cause the dreadful diseases. The metallic contamination like cadmium, zinc, copper, chromium, nickel and other poly chlorinated compounds may cause organ failure in human. It had been reported by earlier researcher that metal like cd, Cu, Fe, Ni, Zn and Pb were higher in sewage affluent irrigated field and

also these metal had been classified as the phytotoxic metals. For the agricultural field which had been irrigated for more than 20 years. The minimum rise in that heavy metals is about 30% & the maximum of 208%. As the Zn is the heavy metal which had raised considerably in the plant and had exceed the limit and its quite phytotoxic. The crop grown on heavy metal containing soil accumulates heavy metal in the plant and is enough to cause the medical health problem in humans as well as the animals which feed on it. It has been observed that rice plant has a tendency to absorb more of the Zn, Cu whereas spinach accumulate Zn, Ni & Cu. Whereas Fe & Mn absorption is less. Long term consumption of unsafe food stuffs like vegetables, fruits, & staple foods may lead to the numerous changes in biological and biochemical cycles in human body whereas accumulate of some of the heavy metals like As, Cd, Cr & Pb are carcinogenic whereas Hg & pb are associated with embryo fetal development normality in children.

Just like the other metal the heavy metals are also found on earth at different level of the crust of the earth. The human activities are the main reason for the discharge of the heavy metals in the lakes, river, seas and oceans. These heavy metals had been reason for the many discuss and for most the reason at carcinogenesis. Even through the mechanism not yet prove but the experiment conduct by the various researcher throughout the world do have conclusive evidences that the heavy metals are also one of the main reason for carcinogenesis. These heavy metals are xeno toxic and affect the replication of the chromosome causes apoptosis and increased in rate of hereditary mutagenesis. Epidemiological studies too have proved that it causes various types of cancer. Some of the heavy metals like Zn & Pb affects the methylation of DNA and acts on the CpG islands and affects imprinting and gene regulations. The heavy metals affect the methylation i.e. it may promote the hypo methylation to hyper methylation and vice versa and thus triggering the carcinogenesis. This happens also due to enzyme inhibition. In-

ternational agency research for cancer and national toxicology programme had mention that some heavy metals in lead carcinogenesis like Pb , Zn, Cu, Ni, Cd. This conclusion results by epidemiological and experimental studies on liver of human and animals. Much of the work has been causes of the cancer and it had been targeted the mutation of the genome. It had been said the epigenetic mechanism are directly related to the genotoxicity of the heavy metals.

In our experiment we have taken two heavy metals for study, Pb (lead acetate) & Zn (zinc sulphate). The lead is the metal that has caused various genetic disfunction, change in the biochemical cycle and change in the physiology of the cell. Thus encouraging the cell to become abnormal that is cancerous in nature. Pb causes many chromosomal aberration, genetic and hereditary disfunction. It has been found that this Pb acetate initiates both the single strand as well as double strand break in the DNA also disturb the interaction between the transcription and translation and interact with the DNA cross linking. It has very harmful effect on nervous system & excretory system. It inhibits the enzyme interaction affects secondary messenger, and the fidelity of the DNA replication is affected. Hence it is one of the most hazardous heavy metal affects to human health. Zn in the high dose or bio magnification of the Zn at cause the similar effect as that of the cadmium, an another heavy metals. Zn cause necrosis and it is hepatotoxic to liver. Zn also affects to skin and kidney.

MATERIALS & METHOD:

The onion was purchased from the vegetable market. Three pots were used for growing onion instead of mud sand was used. It was washed & autoclaved as well 0.1 M solution of Pb acetate was used for the experimental pot no. 1, 0.1M solution ZnSO4 was used on the experimental pot no.2 and double distill water was used for pot no.3 as a control. The root was grown till the root reached the length of 2 inch. Onion root tip 1 to 2 cm were incubated with choline (0.05%) and then was fixed with Conroy's fixative for 8 hours. Then the fix root tips were incubated with 1N HCL at 60°C for 10-15 minutes. After every treatment the root was washed with H₂O two to three times. The treated root tips were stained for 10- 15 minutes and squash was prepared on the microscopic slide. The prepared slide were examined under a compound microscope under oil immersion lens. Photomicrograph were taken from the well spread preparation and all the measurement were taken using the software images.

RESULTS & DISCUSSION:

As per the table no 1, it is clear that the chromosome no of Allium cepa was 16. The total chromosome length (TCL) had been 200 and the average chromosome length (ACL) is found to be 12.5 in table no 1. The sample 1(lead acetate) quadrant observation was taken it was found that P small arm and Q long arm ratio varies considerably it ranged from 0.78 to 0.91.

The sample 2 (zinc sulphate) shows the more variety in the arm ratio. It ranges from 0.44 to 0.78.

The sample 3 (control) shows almost similar arm ratio 0.80 to 0.92 except the last reading showed 0.582 reasons not known.

In table no 3 the chromosome type i.e. subtelomeric (ST), submetacentric (SM), metacentric (M) show different result. The sample treated with the lead acetate has all the chromosome as metacentric whereas the sample treated with ZnSO4 shows that 4 chromosome was metacentric, 8 was submetacentric chromosome, 4 was subtelomeric. The control showed that 12 chromosome was metacentric and 4 was submetacentric and no chromosome was subtelomeric.

Table 1

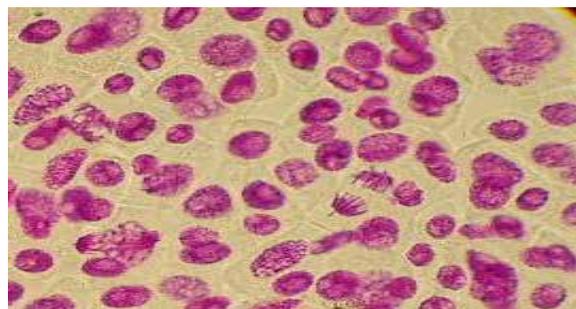
Sample no	Chromosome number	TCL	ACL
1 (treated with lead acetate)	16	200	12.5
2 (treated with zinc sulphate)	16	200	12.5
3 (control)	16	200	12.5

Table 2

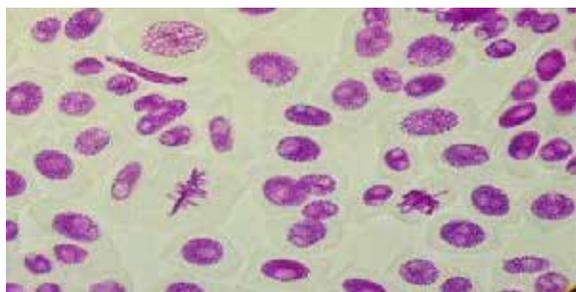
Sample no	Chromosome number	P small arm	Q long arm	Total	AR
1	16	26.90	29.28	56.18	0.91
		22.96	29.28	52.24	0.78
		22.90	27.34	50.24	0.83
		19.02	22.34	41.36	0.85
2	16	9.37	20.87	30.24	0.44
		9.93	16.15	26.09	0.61
		10.91	15.00	25.90	0.72
		7.83	9.95	17.77	0.78
3	16	13.89	15.88	29.77	0.87
		13.04	16.19	29.23	0.80
		10.08	10.89	20.97	0.92
		6.97	13.06	20.02	0.582

Table 3

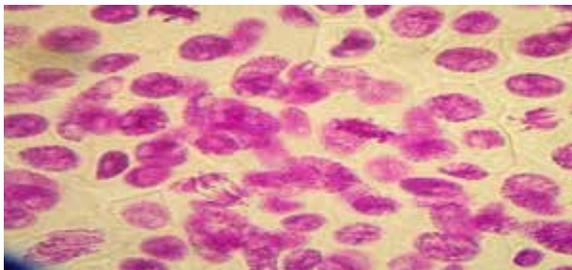
Sample no	Chromosome number	Chromosome type		
		MT	SM	ST
1	16	16	0	0
2	16	4	8	4
3	16	12	4	0



SAMPLE NO. 1



SAMPLE NO. 2



SAMPLE NO.3

CONCLUSION:

The decline of fresh water has forced the law maker to use sewage water for irrigation. As a result the periurban agricultural fields are using the sewage water for the irrigation. Thus prompting WHO to come up with the parameters and scale for the quality of the treated sewage water. The sewage irrigation not only changes the soil chemistry but also the underground

water quality, as well as make agricultural land breeding ground many harmful and dreaded diseases. The pathogenic micro-organism may affect the farmer and the family member and thus may trigger the epidemic in villages. The helminth species like trichuris, hookworm, ascaris and other bacterial like *E. coli* are commonly present. The vegetable is one of the staple diet and an important food for balance diet. These vegetables lead as the part of the plants where most of the heavy metal accumulate. The presence of the heavy metals more than the prescribed scale causes the biomagnification as well as becomes phytotoxic for plants and consumers. The lead causes damage to the kidney and brain and causes miscarriage in pregnant women. Lead also affects the sperm production in males. The zinc has the adverse effect only brain if it reached the particular level. In our experiment it had been shown that heavy metal had caused the mutation in *C. albicans* and thus if high level of the heavy metal present in the vegetables and staple food is consumed by human then it may cause bioaccumulation and consumer may be affected with dreaded diseases.

REFERENCE

- Gahwan, H. K., Ameri, M.Q., Zaidan, K. H., Saadi, A., & Ewadh, J. M. (2012). Heavy metals induce apoptosis in liver of mice, 3 (2), 148-150. | 2. Chakravarty, B., & Srivastava S. (1992). Toxicity of some heavy metals in vivo and in vitro in *Helianthus annuus*, 283, 287-294. | 3. Takiguchi, M., Achanzar, E. W., Li, G. W., & Waalkes, P. M. (2003). Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation, 286, 355-365. | 4. Shah, J. A., Lakkad, C. B., Rao, V. M. (2013). Evaluation of DNA damage induced by lead acetate in human lymphocytes, using comet assay, 2 (10), 1-4. | 5. Yerasai, R. K. P., Reddy, K. S. Y., Reddy, K. G., & Prasad, R. M. (2013). Sewage irrigation can sustain the soil health: a review, 3 (4), 470-472. | 6. Rattan, K. R., Datta, P. S., Chhonkar, K. P., Suribabu, K., & Singh, K. A. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater- a case study, 109, 310-322. | 7. Parashar, P., & Prasad, M. F. (2013). Study of heavy metal accumulation in sewage irrigated vegetables in different regions of Agra district, India, 3, 1-8. | 8. Ensink, J. H. J., Scott, A. C., Broker, S., & Cairncross, S. (2009). Sewage disposal in the Musi-river, India: water quality remediation through irrigation infrastructure, doi 10.1007/s 10795-009-9088-4. | 9. Martin, W., & Griswold, W. (2009). Human health effects of heavy metals, 15. www.engg.ksu.edu/CHSR | 10. Banerjee, A. Wastewater use for agriculture in India: A background review, GIZ- India.