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Comaparative Effects of Three Heavy Metals on Seed Germination and Mitosis of Pearl Millet

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The problem of pollution in our natural resource base has become a major issue in the present world. All organisms are affected by this problem, but the stationary life of plants makes them more prone to damage by a variety of pollutants. Among various pollutants of air, water and soil, heavy metals are significant contributors to intoxication of plants by pollutants. They are present in majority of industrial effluents, agricultural chemicals and even domestic wastes. The plants growing in areas with high level of contamination have developed mechanisms to tolerate the toxicity. One of the mechanisms is to convert the toxic elements into less toxic salts and store them in various tissues. But even this process causes various damages to the plant. The present investigation demonstrates the effects of 3 heavy metals (Pb, Hg and Cd) on germination and early mitotic divisions of pearl millet (*Pennisetum glaucum* (L.) R.Br.). All the three metals were able to elicit a response in form of mitodepression and chromosomal aberrations. The study explores the reasons for the observed clastogeny and mitotic disruption. It also points out towards the reduction in survival as well as abnormal phenotype of the plants if grown in a polluted environment. The study is important since it uses Pearl Millet as the material for bioassay of heavy metals. This plant is considered very hardy and tolerant to various stresses like drought, temperature and salinity. However, heavy metals were able to induce significant abnormalities in its chromosomes, which is a clear cut indicator of their massive toxic potentials.

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1. INTRODUCTION

Metals can be defined as chemicals which can react with acids to liberate Hydrogen, with Hydroxyl ion to form hydroxides and can also act as positive ions in various reactions. The term 'heavy metals,' is used for transition metals, of group 1B to 8B of periodic table. According to Blum [1] "all metals having density greater than or equal to 5 g/cm^3, should be called heavy metals". On the other hand Borovik [2] considered "only non-essential metals as heavy metals". Out of these elements some are essential for plants and animals while others are non-essential. Lead, Mercury and Cadmium belong to this category and are non-essential elements.

According to Prasad [3] since the metallic pollutants are generally non-biodegradable they may be readily absorbed by the plants, and may enter into the food chain easily" [3]. So the metals that enter the food chain through the plants will someday reach the humans as well. As the race for development progresses we are finding higher and higher heavy metal residues in our resources. It is beyond doubt that contamination of our resources has grown with time.

Heavy metals are used in various industries like textile, leather, instrumentation, drug manufacture, chemicals, dyeing, smelters, foundries, electroplating etc. Their effluents have varying amounts of these metals in mostly soluble form. In majority of cases these effluents are released in the nearby water bodies, sewers, or on land. Upon reaching soil or water, these metals associate with different organic ligands and functional groups such as –OH, -PO_3H_2, -NH_2, -COOH, -SH etc [4]. Heavier loads of metals would be expected to induce higher levels of toxicity in the exposed plants.

Besides concentration, another important factor that affects the availability of a particular metal is its pH. According to Skowronski [5]. under lower pH, metals generally tend to form more toxic salts, however under high pH they often get precipitated and hence unavailable. Also under alkaline environment, H+ ion concentration increases which can compete with the cation thus reducing the its toxicity [6]. However Babich and Stotzky [7] are of the view that the toxic potential of some heavy metals may be reduced in one type of environment and increase in another. Thus we can say that solubility of metals is the most important aspect which has to be kept in mind while deciding its toxicity for any organism.

Since, these heavy metals are taken up and stored in the body of plants; they may easily reach the human system through food chain. Thus, the poison that we add to environment finally comes back to us. Out of the different heavy metals, Lead, Mercury and Cadmium have most severe effects on human beings so it was thought to study their effect on plant system to get a view of probable long time effects on animal systems. Since it is very sensitive to even small changes in growth environment, most of the plant-based studies done of heavy metal toxicity use *Allium* as the model. Although it gives good results, use of *Allium* as a model for crops belonging to Graminae (Grain family) cannot be accepted on the fundamental argument that Grasses are sturdy and have exceptional tolerance towards stresses like metallic pollution [8,9]. Thus it was thought worthwhile to consider a grass family crop (Pearl millet) for this study.

2. MATERIALS AND METHODS

During the study, pure selfed seeds of *Pennisetum glaucum* (L.)R BR, were procured from CSAU, Kanpur. The seeds were washed thoroughly under running water for 10-15 min and then soaked for 5 h in distilled water. The soaked seeds were then divided into 16 sets of 25 seeds each in separate petri-dishes. In first five petri-dishes, 5 different concentrations of Lead ($PbNO_3$) were added respectively. Similarly in next five, different concentrations of Mercury ($HgCl_2$) and in last five different concentrations of Cadmium ($CdCl_2$) were added. 16th petri-dish was filled with distilled water and maintained as Control.

For treatment, freshly-prepared aqueous solutions of three heavy metals (Pb, Hg, Cd) in 5 concentrations 10 ppm, 20 ppm, 50 ppm, 100 ppm and 200 ppm prepared by adding 10, 20, 50, 100 and 200 mg of salt respectively to 1 litre volume of distilled water in each case. All chemicals used were of Merck India Ltd, Mumbai (99.2% pure AR grade).

The seeds were soaked in these solutions for 5h. For control, some seeds were soaked for the

same period in distilled water. All the seeds were subsequently washed in running tap water for at least 15 min and germinated in separate petri-dishes (marked by the treatment given) on wet cotton wool. Germination was recorded in each set. When the roots were 4-5mm in size, they were plucked at a specific time and fixed in marked vials having Carnoy's fluid. After 24 hr, they were transferred to 70% ethanol and stored at $4^{O}C$ for cytological analysis.

For the cytological analysis, the fixed roots were first hydrolyzed in 1N HCl at $60^{O}C$ for 15 min. The roots were subsequently washed in distilled water and stained with 2% acetocarmine for 45 min. Slides were prepared using the standard squash technique. A total of 5 root tips were analyzed for each set, thus studying on an average 500 - 600 cells / set.

3. RESULTS AND DISCUSSION

3.1 Germination Studies

Table 1 gives the data of Germination percentage. It was observed that the germination in Control was 92.32%. A clear cut dose based reduction in germination percentage could be observed in all three test metal treatments. However, maximum reduction in germination was brought about by Mercury where the highest dose (200ppm) brought down the germination to 23.84%. Cadmium was less damaging at lower doses but at the higher ones it showed a germination depression almost similar to Lead (Fig 1).

3.2 Cytological Investigations

Table 1 and Plate 1 give an account of various anomalies induced by heavy metal treatments in pearl millet. With a general Active Mitotic Index (AMI) of 11.54%, the controls showed almost perfect mitosis. The higher doses of all the tested metals induced significant drop in AMI. The AMI reached down to 4.26% (200 ppm $PbNO_3$), 2.98% (200 ppm $HgCl_2$) & 4.72% (200 ppm $CdCl_2$).

Together with decrease in AMI, there was a simultaneous increase in the total abnormality percentage reaching the highest number (28.86%) at 200 ppm $HgCl_2$.The treatment of various concentrations of mercuric chloride was able to induce anomalies related to spindle dysfunction and physiological behaviour of chromosomes. Lower doses were marked by spindle anomalies while higher doses recorded greater number of physiological anomalies. Spindle anomalies were characterized by unorientation, scattering and precocious movement of chromosomes. None of these were visible at 100 and 200 ppm doses and reached their maximum values at 50 ppm dose. Stickiness and clumping although present at lower doses also, were particularly significant at 100 and 200 ppm doses. Laggards were common at all doses as was clumping at anaphase. Disturbed polarity and chromatin bridges were however, uncommon.

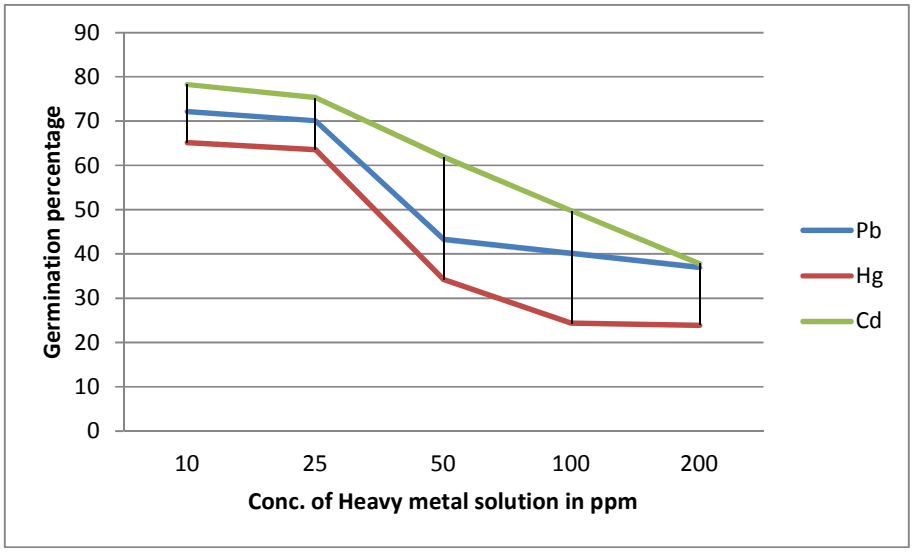

Fig. 1. Germination % of seeds under different treatments

Behaviour of $PbNO_3$ treated cells, was similar to $HgCl_2$ treated ones but here spindle anomalies were common at all the doses. Stickiness reached a maximum value of 4.76% at 200 ppm dose. Clumping was almost equally represented being highest (3.03%) at 200 ppm dose. Laggards were frequent in occurrence and very common at higher doses. Clumping of chromosomes reached a value of 3.90% at the highest dose. Minor occurrence of chromatin bridges and insignificant number of cells with disturbed polarity, could be seen.

In $CdCl_2$, spindle anomalies were well represented.but the difference was in their frequency. While unorientation reached a maximum of 5.08% at the highest dose of $CdCl_2$. Scattering was common. Large number of cases of precocious movement and fragmentation were encountered. Stickiness at metaphase was observed to be reaching a high of 3.81% $CdCl_2$ (200 ppm) treatment. Anaphasic aberrations were dominated by laggards maintaining more or less equal presence in both. Disturbed polarity was common in case of $CdCl_2$. Clumping at anaphase reached only 1.69% at 200 ppm $CdCl_2$.

Analysis of cytological behaviour under the influence of different heavy metals, provides us with a valuable tool for assessing the genotoxic potentialities. This study on one hand gives us information about the effect of the toxins on numerical increase in the cells (cell division) and on the other; it provided an insight into the mechanism of action of each toxin.

Germination requires heavy expenditure of energy. A drop in the germination percentage at various treatment doses might indicate that the heavy metals interfere in the respiratory pathways thus reducing the accumulation of ATP. This assumption is further strengthened by the fact that “germination and cell division are inhibited by various agents that suppress the energy processes like glycolysis and formation of Acetyl CoA” [10].

As far as the effects of heavy metals on normal cell division are concerned, it was observed that all these were able to depress the AMI even when used in low doses. A drop in AMI indicated that these interfere in the normal sequence of events in the mitosis, thus preventing a number of cells in interphase from entering into prophase. “Such interference might be a result of inhibition of DNA synthesis”, [11]. Epel [12] reported that “the rate of mitosis was closely related to resultant levels of ATP and mitosis could be blocked at any stage at appropriate time by adding optimum concentration of respiratory inhibitors”.

A clear-cut predominance of Metaphase over other phases could be observed in almost all the treatment sets. It appears that there might be some factors that tend to arrest the cell cycle at metaphase. Cummins et al. explained this by saying that there are some proteins, which are responsible for transition from metaphase to anaphase, by transformation of chemical energy into mechanical energy. However when respiration is partly inhibited by heavy metals, then a lower amount of chemical energy (ATP) is available to these protein and don’t function properly. This in turn causes metaphase arrest and a consequent increase in metaphase abnormalities.

A reduction in mitotic activity with increase in cytological anomalies seems to be common effect of most heavy metals on different plants [13-16].

In the present investigation, mitotic anomalies related to chromatin stickiness, spindle dysfunction, chromosome fragmentation and abnormal association were observed. Chromosome stickinessand clumping were significantly higher than most other anomalies. These may be the result of interaction, of positively charged metal ions with non-histones and DNA, which may result in clumping and stickiness [17]. McGill et al. [18] and Klasterska et al. [19] have attributed stickiness and clumping to “improper folding of chromatin fibers leading to creation of sub-chromatid bridges between the chromosomes”. Jayabalan and Rao [20] have attributed stickiness to “changes in cytochemically-balanced reactions, which in turn lead to changes in cytoplasmic viscosity”.

Chromosome fragmentation was observed in almost all sets at higher doses. Sharma and Sharma [21] suggested that “the upsetting of nucleic acid metabolism ultimately results in disturbed protein re-duplication causing chromosomes to break at several loci and form fragments”. However Evans [22] assigned fragmentation to “the failure of broken chromosomes to recombine or due to mis-repair of DNA”. However no definite mode of action for causing fragmentation can be given to the test heavy metals.

Scattering of chromosomes can be an effect of the breakdown or inhibition of spindle system. “It can be said that the signal given after prophase for organization of spindle may have been blocked” [23]. It has also been observed that attachment of some metal ions with the Sulfhydryl groups of the spindle fibres, makes the monomers unfit for polymerization to form fibres [24] Similarly, precocious movement, another spindle anomaly can be attributed to spindle dysfunction [25].

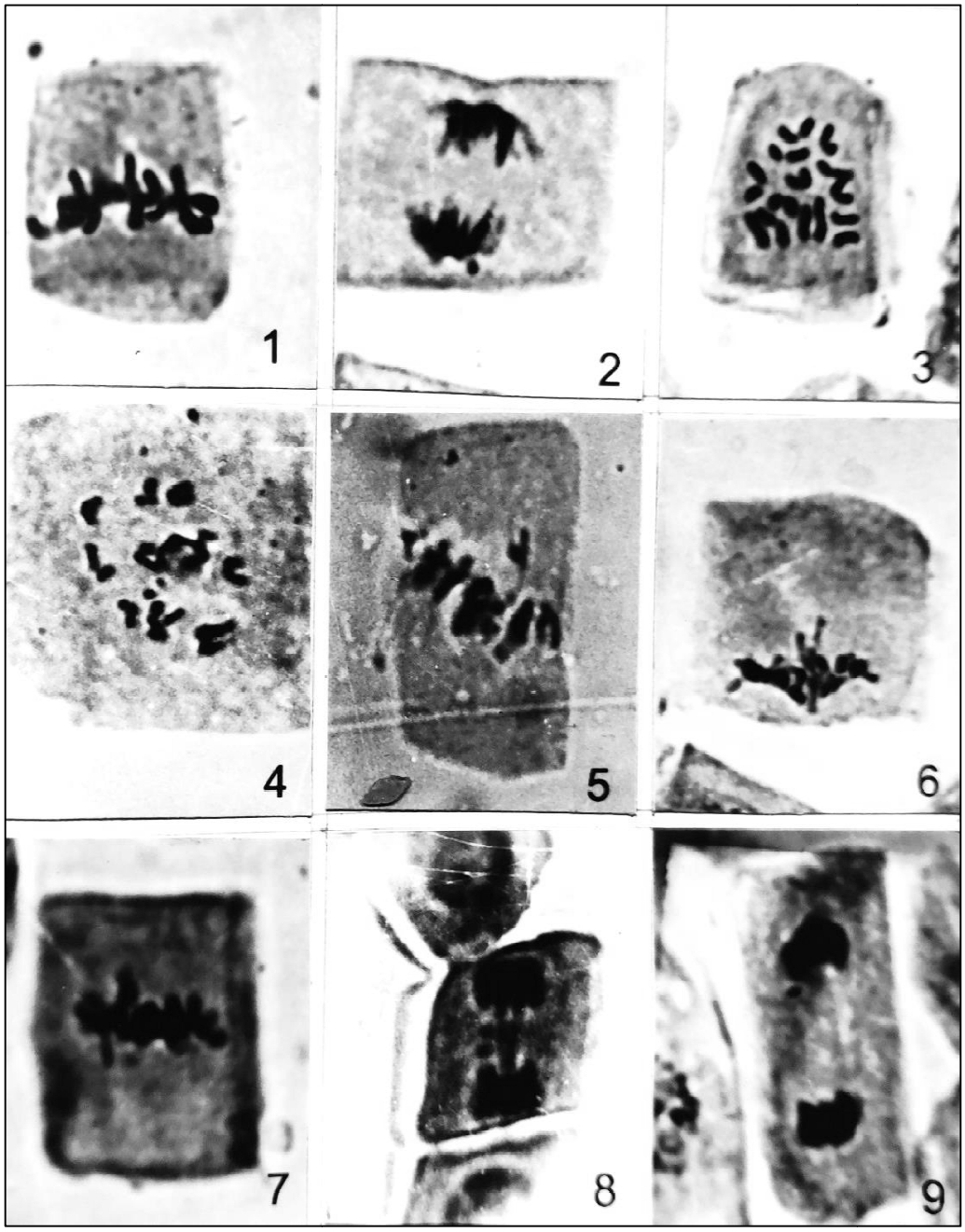

Plate 1. Mitosis and Control and Treated Cells of Pearl Millet.

1=Normal Metaphase; 2=Normal Anaphase; 3=Unorientation at Metaphase; 4=This looks like late prophase, not metaphase? Fragmentation at Metaphase; 5=Precocious Movement; 6=Disturbed Polarity; 7=Stickiness at metaphase; 8=Sticky Anaphase with bridge; 9=Telophase with laggard

Table 1. Major chromosomal anomalies induced by the 3 heavy metals in mitosis of pearl millet

Treatment (ppm)	GP	AMI (%)	TAb	Metaphase abnormalities (%)						Anaphase abnormalities (%)				Others (%)	TAb (%)
				Un	Sc	Pm	Fr	St	Cl	Lg	Dp	Br	Cl		
CONTROL	92.32	11.54	5	0.35				0.52							0.86
Lead (as $PbNO_3$)															
10	72.16	9.02	15	0.44	0.22			1.11	0.22	0.88			0.44		3.32
25	70.08	8.76	30	1.15	0.46	0.23		1.61	0.69	1.38		0.23	0.92	0.23	6.85
50	43.36	5.42	23	0.37	1.11			2.22	1.48	1.11			2.22		8.49
100	40.16	5.00	37	1.20	1.60	0.80	0.40	3.20	2.40	2.00	0.80	0.40	2.00		14.80
200	36.96	4.62	50	0.43	2.16	0.86	1.73	4.76	3.03	3.46		0.86	3.90	0.43	21.64
Mercury (as $HgCl_2$)															
10	65.12	8.14	20	0.74	1.47	0.49		0.98		1.23					4.91
25	63.52	7.94	22	0.75	1.50	0.50	0.25	0.75	0.25	1.25			0.25		5.54
50	34.24	4.28	28	1.87	3.27	0.93	0.47	1.40	0.47	2.80		0.47	1.40		13.08
100	24.32	3.04	29				2.63	5.92	1.97	3.29		1.31	3.95		19.08
200	23.84	2.98	43					6.71	10.07	4.03			8.05		28.86
Cadmium (as $CdCl_2$)															
10	78.24	9.78	12	1.02	0.20			0.61			0.41			0.20	2.45
25	75.36	9.42	30	1.70	1.06	0.42		1.49		0.85	0.64	0.21			6.37
50	61.92	7.74	25	1.81	1.03	0.52		1.55	0.52	0.77	0.26				6.46
100	49.76	6.22	48	2.89	1.93	1.29	0.96	2.25	1.29	1.61	0.96	0.64	1.29	0.32	15.43
200	37.76	4.72	49	5.08	2.97	2.12	1.27	3.81		3.39		0.42	1.69		20.76

GP=Germination Percentage; AMI=Active mitotic Index; TAb=Total number of abnormal cells; Un=Disorientation – dis rather than un of chromosomes; Sc=Scattering of chromosomes; Fr=Fragmentation of chromosomes; Pm=Precocious movement of chromosomes from the Metaphase plate; St=Stickiness of chromosomes; Cl=Clumping of chromosomes; Lg=Lagging chromosomes; Dp=Disturbed polarity of chromosomes; Br=Chromatin bridge between the poles; Tab (%)=Total percentage of abnormal cells

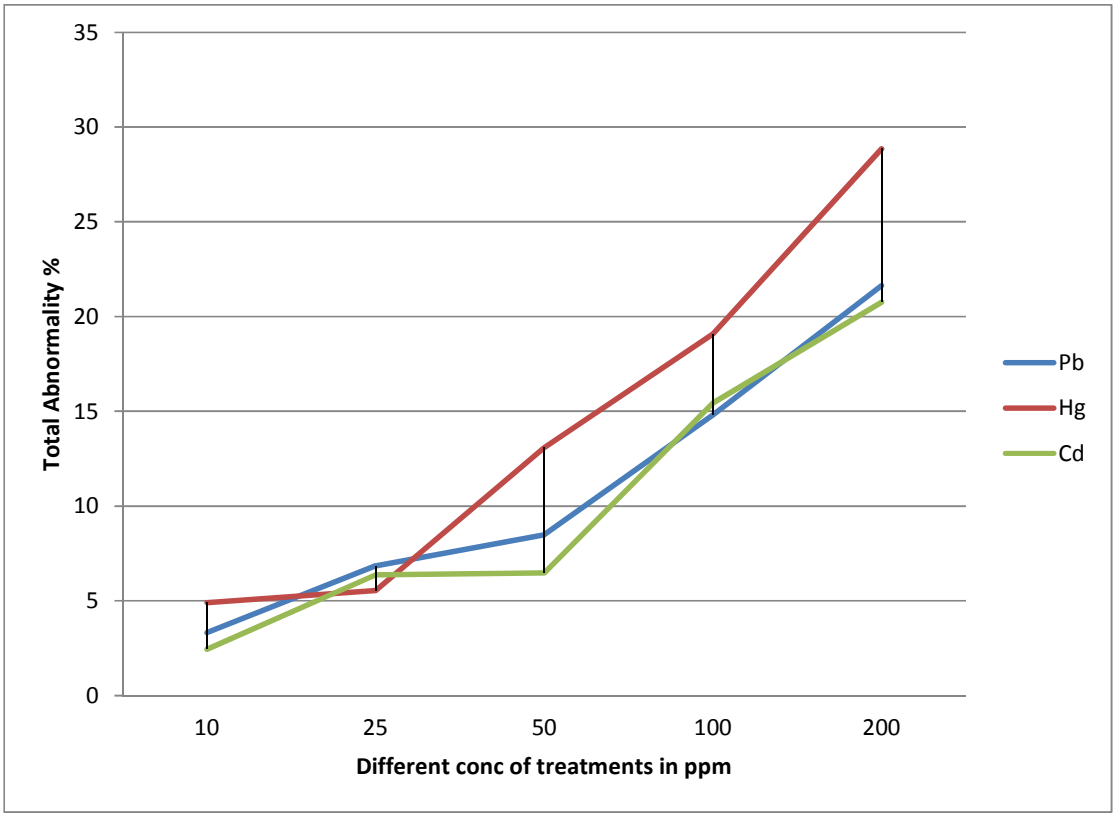

Fig. 2. Total Abnormality % induced by various treatments

Laggards at Anaphase are usually thought of as spindle abnormalities but Gomez-Arroyo and Villiobos-Pietrini [26] used the term 'chromosomes with inactivated centromeres' for lagging chromosomes. According to them, this anomaly occurs due to absence of centomeres or due to inability of centromere to condense microtubules.

Chromatin bridges appear to be the results of stickiness and failure of chromosomes to separate properly. "Bridges might also be viewed as indicators of exchange between the chromosomes involving breakage and proximal reunion" [27].

Among other abnormalities were chromosome erosion, multipolarity, polyploid cells etc. Micronuclei at Telophaseprobably represent the remnants of laggards and fragments of earlier phases, which fail to reach the poles [28].

4. CONCLUSION

It can be concluded that all the three heavy metals viz. Pb, Hg and Cd elicited mitodepressive, genotoxic and clastogenic effects on the root tips of Pearl millet. Similar results have been obtained by various workers for germination of seeds under the influence of heavy metals [29-36].

Some workers have suggested that heavy metals like Pb are able to reduce the germination percentage because of respiratory blockage [37,38]. It is also well known that these metals occur in industrial effluents that reach our fields directly or through contaminated water bodies and enter into the crop plants. There they produce various ill effects that would reduce the growth and yield of crops [39]. The major problem arises when the crop plants start to accumulate these metals it their tissue systems and are then transferred to animals and humans. Chromosomal aberrations observed in plant cells suggest that a similar reaction could occur in animal cells as well [40,41]. Sensitivity of the roots of the test plant towards concentrations of heavy metals ranging from 10 to 200 ppm is suggestive of similar effects occurring at contaminated sites where concentrations may range from 50 to 150 ppm [42].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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