CHAPTER–6

SUMMARY AND CONCLUSION
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SUMMARY

The salient features of the present investigation are summarised bellow in a nutshell under specific category of study.

6.1 INFLUENCE OF HEAVY METALS ON GROWTH AND DEVELOPMENT:

6.1.1 Influence of lead on growth and development:

Lead did not exhibit much influence on growth index at lower concentrations of 50 and 100 mg kg\(^{-1}\) of soil treatment during the period of first harvest in winter. However, at higher concentrations there was decline in growth. The growth index of control plant was 54.17, while at 50mg kg\(^{-1}\) of soil, there was marginal increase i.e. 56.63 which subsequently dropped to 43.73 at 500mg kg\(^{-1}\) treatment.

Growth was maximum at the time of 2\(^{nd}\) harvest in summer with a growth index of 69.47 for control followed by spring (4\(^{th}\) harvest) with growth index of 64.77 and autumn (3\(^{rd}\) harvest) with 57.78. With the passage of time the impact of lead was found decreasing considerably which was indicated by the fact that in spring (4\(^{th}\) harvest) growth indices for different treatments were close to control.

6.1.2 Influence of mercury on growth and development:

Compared to lead, mercury was found little more toxic for growth and development, which was indicated by the fact that growth indices for different doses of mercury were little lower than their corresponding values for lead. However, like lead, in case of mercury also at lower concentrations growth was similar or slightly more then that of control. However, at higher concentrations there was a gradual decline. Growth indices for all the treatments at the time of 1\(^{st}\) harvest (in winter) varied from 53.33 to 37.00 against 55.62 to 43.75 in case of lead treatment.
The impact of seasonal variations was similar to that of lead. But in case of mercury from 3rd harvest (in autumn) onward lower concentrations did not exhibit much variation in growth indices; however at higher concentrations the effect was quite significant.

6.1.3 Influence of cadmium on growth and development:
Cadmium proved to be far more toxic than that of lead and mercury and concentrations above 200mg kg⁻¹ proved fatal. Even at 200mg kg⁻¹ of soil treatment measurable growth was found in winter and summer; thereafter the plants did not survive. Cadmium caused severe reduction of growth in case of all the treatments. At the time of 1st harvest in winter growth index at 50mg kg⁻¹ treatment was 31.66 against 54.17 in control while at 100mg kg⁻¹ treatment there was recorded nearly three times decline in growth. By and large, the impact of seasonal variations was in the line of lead and mercury. Cadmium not only severely retarded growth but also caused morphological deformation. The leaf sizes were considerably reduced and the plants exhibited stunted growth, particularly at 100 and 200mg kg⁻¹ treatment.

On the whole, all the three heavy metals caused gradual decline in growth index with the increase of doses; although in case of lead and mercury lower doses had little impact. The order of toxicity on growth and development could be represented as follows:

\( \text{Cd} > \text{Pb} > \text{Hg} \).

Seasonal variations considerably influenced growth which was at per with higher doses of metal or even more. Summer was found to be the best season for growth while in winter very poor growth was recorded. The order of the adverse affect on growth in case of all seasons are as follows:

\( \text{Winter} > \text{Autumn} > \text{Spring} > \text{Summer} \).
6.2 IMPACT OF HEAVY METAL ON OIL CONTENT:

6.2.1 Impact of lead on oil content:
Due to lead oil content was marginally enhanced at lower concentration of 50mg kg\(^{-1}\) against all seasons and occasionally at 100mg kg\(^{-1}\) of soil treatment also. However, beyond that there was a decline with increase in concentrations of lead which was statistically found to be significant.

Seasonal variations caused significant changes on oil content. Summer was the best season in the sense that during summer upto 200mg kg\(^{-1}\) treatment, oil content was little higher than that of the control. However, beyond that there was a declining trend. On the whole, summer and spring were best seasons with 0.618% and 0.640% oil content in case of control respectively, lowest value being 0.540% corresponds to autumn.

6.2.2 Impact of mercury on oil content:
In case of mercury, however seasonal variations considerably influenced the oil content due to different doses applied. At the time of 1\(^{st}\) and 2\(^{nd}\) harvest during winter and summer respectively, oil contents was higher than the control in all the treatments which were statistically significant. For example, in winter (1\(^{st}\) harvest) against 0.575% in control oil content was 0.713% at 50mg kg\(^{-1}\) of treatment. This observation was remarkable as this was not observed in case of lead and cadmium. However, after 3\(^{rd}\) and 4\(^{th}\) harvest in autumn and spring oil content was lower for all the treatments than that of control. However, like lead in case of mercury also as a whole there was a declining trend with increase in concentrations except a few cases.

6.2.3 Impact of cadmium on oil content:
Cadmium had severe effect on lemongrass and beyond 200mg kg\(^{-1}\) soil treatment all plants died. Oil content could be estimated upto 50 and 100 mg kg\(^{-1}\) treatment only. There was recorded measurable growth against 200mg kg\(^{-1}\) treatment only in summer and hence oil
content was estimated for summer, subsequently the plant died. At the time of 1st harvest in winter oil contents were 0.61% and 0.603% against 50 and 100 mg kg\(^{-1}\) treatment respectively which were little higher than control. At the time of subsequent harvest, oil contents were comparable or slightly lesser than the corresponding control.

On the whole, heavy metals caused decline in oil content, particularly at higher concentrations, although the impact was not uniform. At lower concentrations either the oil contents remain unaffected or there was little enhancement over the control.

The toxic impact of heavy metals on oil content could be summarised in the following order.

**Cadmium>Lead>Mercury.**

### 6.3 IMPACT OF HEAVY METAL ON QUALITY OF LEMONGRASS OIL:

Oil quality was assessed by Gas Liquid Chromatography. Of the 48 compounds detected as constituents of the oil, six most important compounds were taken into consideration namely geranyl acetate, citral-a, citral-b, geraniol, linalool and cineole. Although most common cultivars of lemongrass contain citral-a and citral-b in the ratio of approximately 2:1 as major constituent of oil and account for over 80% of the total constituent. The particular mutant used in the study contain geranyl acetate as major constituent which constitute over 75% of the oil and hence percentage of geranyl acetate was considered as quality index.

#### 6.3.1 Impact of lead on oil quality:

Lead caused considerable decline in oil quality. Against 76% of geranyl acetate in control, it declined from 66.9% at 50 mg kg\(^{-1}\) to 44.8% at 350 mg kg\(^{-1}\) treatment. This is unlike the impact of lead on growth and oil content.
6.3.2 Impact of mercury on oil quality:

Mercury also caused decline in oil quality but compared to lead the decline was to lesser degree. At 50 mg kg\(^{-1}\) soil of mercury geranyl acetate was 73.5% which dropped to 66.9% in 500 mg kg\(^{-1}\) treatment.

6.3.3 Impact of cadmium on oil quality:

In case of cadmium, although it was most toxic on growth and oil content yet its impact on oil quality was minimum. Of the two surviving batches of plants, namely 50 and 100 mg kg\(^{-1}\) treatment, geranyl acetate levels were 76.4% to 75.6% against 76% in control. This shows that cadmium did not affect oil quality unlike lead and mercury.

6.4 PROLINE ACCUMULATION DUE TO HEAVY METALS STRESS:

Proline accumulation is a well established biochemical indicator for plants in response to water stress and plant try to withstand and overcome drought condition by accumulating proline. The present study showed that apart from this general trend lemongrass response to heavy metals stress by proline accumulation.

Proline accumulation was estimated against short term exposure to heavy metal (2 months after transplantation) and long term exposure (nine months after transplantation).

Proline content was estimated separately in old and newley emerged leaf. In new leaves, proline level was found to be considerably higher then old leaves in all the cases. In control (two months after transplantation) proline level in new leaf was 0.805 \(\mu\) moles g\(^{-1}\) tissue. This is understandable as new leaves are metabolically most active while in older leaves proline and other amino acids get polymerised into protein to varied extent.

6.4.1 Impact of lead on proline accumulation:

In case of lead proline level at lower concentration (50 mg kg\(^{-1}\)) was nearly same as that of control. However, beyond that there was concomitant increase in the level of proline and
in case of higher dose of lead (500mg kg\(^{-1}\)) proline level was 2.090 and 4.567 \(\mu\) moles g\(^{-1}\) tissues for old and new leaves respectively. This was nearly four times increase for old leaves and more than five times increase for new leaves than the control.

In case of long term exposure similar trend was observed with marginal increase for most of the treatments compared to their corresponding values for short term exposure. Proline level was found to be 3.984 and 5.247 \(\mu\) moles g\(^{-1}\) tissue for old and new leaves respectively against 500mg kg\(^{-1}\) of soil treatment.

6.4.2 Impact of mercury on proline accumulation:
Over all trend was similar to that of lead in case of mercury also. However, for mercury proline levels were considerably higher than their corresponding values for lead. For short term exposure at 50mg kg\(^{-1}\) of mercury proline levels were 1.505 and 1.968 \(\mu\) moles g\(^{-1}\) tissue for old and new leaves which was 2 and 3 times higher respectively than control.

However, following long term exposure proline levels were slightly lower than that of shot term exposure, but much higher than control. Moreover, the variations due to different doses of mercury following long term exposure were not pronounced.

6.4.3 Impact of cadmium on proline accumulation:
Cadmium also resulted in higher accumulation of proline following short term exposure. Proline level at 50mg kg\(^{-1}\) of soil was 1.7 to 8 and 2.032 \(\mu\) moles g\(^{-1}\) tissue for old and new leaves which were higher than the corresponding values for lead and mercury. However, data could be generated for upto 200mg kg\(^{-1}\) of soil treatment. Beyond this cadmium proved fatal.

Unlike lead and mercury following long term exposure at lower concentration of 50mgkg\(^{-1}\) of soil treatment proline level increased. However, at 100 and 200mg kg\(^{-1}\) of soil proline levels significantly declined.
6.4.4 Proline accumulation in root due to heavy metals:

Proline level in root was observed after long term exposure as roots could not be disturbed during the course of experiment.

In case of lead at 50mg kg\(^{-1}\) treatment proline levels were 0.527\(\mu\) moles g\(^{-1}\) tissue against 0.425\(\mu\) moles g\(^{-1}\) tissue in control which rose to 1.416 at 500 mg kg\(^{-1}\) treatment. There was a concomitant rise in proline level with increasing doses of lead, but compared to leaves the level was much lower.

The trend was similar for mercury to that of lead but the level was lower than that of lead. The range of variation was 0.337 to 0.937\(\mu\) moles g\(^{-1}\) tissue against 50 and 500 mg kg\(^{-1}\) of soil treatment respectively.

Cadmium stimulated proline accumulation in root which was higher than both lead and mercury. The range of variation was 1.107 to 2.085 \(\mu\) moles g\(^{-1}\) tissue against 50 and 200 mg kg\(^{-1}\) of soil.

The observations reflect that lemongrass responds to heavy metal stress by accumulating proline, in a dose dependent manner. Leaf accumulation was higher compared to root and this tendency for accumulation persist even after long term exposure.

6.5 NITRATE REDUCTASE ACTIVITY AS INFLUENCED BY HEAVY METALS:

Nitrate reductase is the key enzyme in the process of nitrogen accumulation and is associated vitally with the growth and development of plant. Influence of heavy metals stress on nitrate reductase activity was also a prime focus in the present study.

Nitrate reductase activity (NRA) in old and new leaves was measured separately. Moreover, like proline, NRA was measured at two levels i.e. short term exposure (after 2 months) and long term exposure (after 9 months). NRA in old and new leaves were 3.34
and 2.242 μ moles h⁻¹ g⁻¹ fresh weight respectively in control against short term exposure while for long term exposure the corresponding values were 3.056 and 2.751 μ moles h⁻¹ g⁻¹ fresh weight respectively. Hence, under normal condition NRA in old leaves was more than new leaves.

6.5.1 NRA due to lead treatment:
Due to lead following short term exposure NRA was similar or slightly higher than control for lower concentrations of lead. Thus at 50 mg kg⁻¹ of lead NRA was 3.404 and 3.573 μ moles h⁻¹ g⁻¹ fresh weight for old and new leaves respectively which was little higher than control. However, at higher concentrations NRA declined only to a limited extent with 2.106 and 2.400 μ moles h⁻¹ g⁻¹ fr.wt. for old and new leaves against 500 mg kg⁻¹ of soil treatment.

A notable observation was that there was not much difference in NRA between old and new leaves. Following long term exposure similar trend was observed, while at lower concentrations NRA was at par with the control, in higher concentrations the same dropped slightly.

6.5.2 NRA due to mercury treatment:
NRA due to mercury was found to be similar to lead. Following short term exposure NRA at 50 mg kg⁻¹ of soil treatment was 3.347 μ moles h⁻¹ g⁻¹ fr.wt. which was marginally more than control. Beyond this level with increase in mercury concentrations there was a gradual decline in NRA, although there was not much differences recorded among the higher concentrations.

Compared to lead in case of mercury, NRA was lesser than their corresponding values for lead. Following long term exposure in case of lower concentrations, NRA was at par with control like short term exposure. However, unlike short time exposure in case of higher
concentrations, the plants recovered to restore NRA to nearly same level as that of control, indicating that after long term exposure lemongrass can overcome the mercury stress against NRA.

6.5.3 NRA due to cadmium treatment:

Cadmium significantly lowered NRA at lower concentrations of 50mg kg\(^{-1}\) with 2.400 and 1.494 \(\mu\) moles h\(^{-1}\)g\(^{-1}\) fr.wt. in new and old leaves respectively following short term exposure. However, at 100 and 200 mg kg\(^{-1}\) treatment there was a further decline

Following long term exposure, the NRA was higher for all the doses of cadmium than their corresponding values for short term exposure. Moreover, the NRA for all the treatments were comparable to control indicating that prolonged exposure restored NRA and stimulated the plant to overcome cadmium stress.

6.5.4 NRA in root due to heavy metal:

NRA of roots was measured after long term exposure. Overall, in case of lead NRA varied from 1.098 to 0.974 \(\mu\) moles h\(^{-1}\)g\(^{-1}\)fr.wt. against 2.513 \(\mu\) moles h\(^{-1}\)g\(^{-1}\)fr.wt. in control exhibiting a significant decline.

In all treatments of mercury, NRA in roots varied from 1.208 to 0.871\(\mu\) moles h\(^{-1}\)g\(^{-1}\)fr.wt. reporting slightly lower NRA compared to lead.

Cadmium caused maximum decline in NRA in root. The range of variation was 0.985 to 0.871 \(\mu\) moles h\(^{-1}\)g\(^{-1}\) fr.wt. against 50 to 200 mg kg\(^{-1}\) treatment respectively. Beyond this cadmium proved fatal to the plants.

On the whole, compared to leaf in case of root there was a significant decline of NRA for all the three heavy metals against all the concentrations. Unlike leaf, even at lowest concentration of metal there was a significant reduction in NRA of root.
6.6 ACCUMULATION OF HEAVY METALS IN DIFFERENT PARTS OF LEMONGRASS:

Heavy metal accumulation levels were estimated by ICP and AAS for stem, root and leaf. Estimation was done following long term exposure (11 months after transplantation) of all other observations. In case of leaf estimation was done at two levels—short term exposure before 1st harvest (3 months after planting) and long term exposure at the time of 4th harvest (11 months after planting).

After short term exposure, lead accumulation in leaf varied from 148.6 to 324 \( \mu g \ g^{-1} \ dm \) with concomitant increase in soil. The accumulation profile remained similar after long term exposure. However, the lead level was lower than that of short term exposure and varied from 148.6 to 280 \( \mu g \ g^{-1} \ dm \). Beyond 200 mg kg\(^{-1}\) treatment there was a sharp rise in lead accumulation. Highest accumulation was found in root, where lead level varied from 1000 to 5000 \( \mu g \ g^{-1} \ dm \), signifying nearly six to sixteen times increase over leaf accumulation. Like leaf, in both stem and root there was a gradual rise in accumulation with increase doses of lead in soil.

In case of mercury following short term exposure accumulation in leaf varied from 155 to 288 \( \mu g \ g^{-1} \ dm \), which was little lesser than the corresponding values for lead; however, the pattern was similar. Following long term exposure accumulation was little lower than that of short term exposure and varied from 130 to 304 \( \mu g \ g^{-1} \ dm \). Unlike leaf, mercury accumulation in stem was far greater than that of lead which was varied from 552 to 1741 \( \mu g \ g^{-1} \ dm \). The accumulation in stem was more than two times than lead. Mercury was maximum in root and varied from 1329.5 to 3521.4 \( \mu g \ g^{-1} \ dm \), which was nearly nine to
twelve times higher compared to leaf. However, mercury level in root was much lower
than that of lead.

Cadmium accumulations in leaf for both short and long term exposure were greater than
lead and mercury. Its level in leaves was 304 to 429.45 μg g⁻¹ dm⁻¹ for short term exposure
against 50 to 200mg kg⁻¹ of soil treatment respectively. Like lead and mercury, cadmium
accumulation in stem was far greater than leaf. However, accumulation in root was highest
for all. Stem accumulation varied from 814.28 to 2200 μg g⁻¹ dm⁻¹, while the values for
root were 2375 to 5862.06 μg g⁻¹ dm⁻¹ which was far greater than their corresponding
values for lead and mercury.

On the whole, accumulation in leaf was minimum and maximum in root with stem nearly
intermediate between two. This indicates that after absorption, major parts were
sequentially retained by root and stem and only a very small fractions were translocated to
leaf which appears to be the reason why aerial parts look unaffected or little affected
except in cadmium.

The accumulation profile for different parts are in the following order

**Root>Stem>Leaf.**

On the other hand, of the three metals, cadmium is preferentially absorbed more than lead
and mercury. The order of preference for absorption and accumulation are

**Cadmium>Lead>Mercury.**