5. DISCUSSION

The results generated from the soil incubation study and pot culture experiments are discussed below.

The first part of the discussion deals with the soil incubation experiment conducted with the aim to observe the changes occurring in DTPA and diacid extractable heavy metals (Zn,Cu and Ni) in soil at 6 and 12 months period after the addition of amendments (FYM, SSP, $CaCO_3$, and FYM+$CaCO_3$) and added metals (Zn,Cu and Ni) under as well as submerged soil moisture condition.

The second part of the discussion deals with the finding emerged from the five pot culture experiments,each with *.B.juneca, B.campestris, B. carinata, B.napus* and *B.nigra* used as test crops. The study is to compare *the Brassica juneca, Brassica campestris, Brassica carinata, Brassica napus* and *Brassica nigra* with respect to accumulation capacity and uptake of heavy metals (Zn, Cu and Ni) by these *Brassica species* to assess than influence of amendments (FYM, $CaCO_3$, SSP and FYM+$CaCO_3$) on the accumulation and uptake of heavy metals by these *Brassica species.*

DTPA extractable for soil Zn as influenced by amendments and added metals are shown in table 3a and fig. 2a indicate that the content of DTPA extractable Zn was significantly increased from control. These results show that $CaCO_3$ was more effective in reducing the availability of DTPA extractable Zn in soils. Other amendments, *viz.* FYM, phosphate and FYM+$CaCO_3$ could not reduce the DTPA extractable Zn significantly in control as well as metal treated pots.

Data presented 6 and 12 months in table 4a,4b and fig. 2a,2b shows that Zn were extracted by diacid from control, FYM and phosphate treated pots, respectively whereas, this extractant could not extract Zn up to measurable limit (flame AAS) from $CaCO_3$ and combination with FYM+$CaCO_3$ treated pots.

Data as given in table 3b and fig. 2b (after 12 months) show the same trend although amount extracted is lesser than amount of Zn extracted in 6 month by DTPA.

When extraction by diacid was studied the values in table 4a, 4b and fig. 2a, 2b indicate that this extractant could not extract Zn up to measureable limit. Same trend was observed after 12 months and amount Zn extracted in the presence of different amendments were lesser and not up to measureable limit.

When amendments were added to the soil, metal concentration in soil was reduced due to immobilization of metal in soil by amendments.

DTPA and diacid extractable Copper content in soil at 6 and 12 months period data given in table 5a, 5b,6a, 6b and fig. 3a, 3b indicate that mean content of DTPA extractable Cu was 12.6 mg kg^{-1} in control pot. These values, it may be observed that the $CaCO_3$ is the more effective amendment to reduce the DTPA extractable Cu in metal contaminated soil. The effect of metal addition was significant and the average content of DTPA extractable Cu was increased from 10.5 to 14.5 mg kg^{-1} in control as compared amended soil. Data of Cu extraction by DTPA 12 months period as indicated in table 5b and fig. 3b in the presence of different anendments after 12 months also follow the same trend as the data in 6 months, although amount of Cu extracted were lesser in 12 months than in 6 months.

The diacid extractable Cu in soils under various treatments (6 months period) are given in table 6a and fig. 3a from control, 20.3 mg kg^{-1} of Cu was extracted by diacid and that in FYM and phosphate (SSP) treated pots were 19.0 and 18.5 mg kg^{-1}, respectively. However, the diacid failed to extract this metal up to analytically measurable limits from the pots. Treated with either $CaCO_3$ or $CaCO_3$ along with FYM as in case of aforesaid extractants, diacid also extracted significantly hogher amount of Cu from the pots where metal was applied.

The fig. 3a, 3b indicates that there was a significant reduction in DTPA extractable Cu compared to diacid in $CaCO_3$ and FYM+$CaCO_3$ treated soils over control.

DTPA and diacid extractable Ni content in soil at 6 months in given table 7a and fig. 4a show that the Ni content as extracted by DTPA from soils treated with different amendments. In control, Ni content was 0.86 mg kg^{-1} and the average content of Ni in pots treated with FYM, phosphate, $CaCO_3$ and FYM+$CaCO_3$ were 1.00, 1.04, 0.67 and 0.78 mg kg^{-1} respectively. Among the treatments $CaCO_3$ was successful in reducing the DTPA extractable Ni in metal contaminated soil followed by FYM+$CaCO_3$. In case of FYM and phosphate (SSP) treated soils, DTPA extracted more amount of Ni compared to control, metal application has significantly increased the DTPA extractable Ni from control 0.58 to 1.16 mg kg^{-1} in pots where the metal was applied.

Extraction of Ni by DTPA after 12 months in given table 7b and fig. 4b follows the same trend as trend represented in the extraction in 6 month. Although amount extracted in 12 months are lesser than in 6 months. It is also clear that amendment $CaCO_3$ is successful in reducing the DTPA extractable Ni followed by FYM+$CaCO_3$. In case of FYM and phosphate DTPA extracted more amount of Ni over control.

Diacid extractable Ni contents in control and amended soils are given in table 8a and fig. 4a (6 months) on an average, 2.28, 2.38 and 2.74 $mgkg^{-1}$ of Ni were extracted by diacid from control, FYM and phosphate treated pots respectively. As in case of Zn and Cu diacid was unsuccessful in extracting any measurable amount of Ni from either $CaCO_3$ or FYM+$CaCO_3$ treated pots. Metal application increased the diacid extractable Ni from control 1.33 to 1.67 mg kg^{-1} in metal applied soils.

Extraction of Ni by diacid as given by data in table 8b and fig. 4b (12months) shows that as in case of Zn and Cu, diacid is unsuccessful in extracting any measurable amount of Ni from either $CaCO_3$ or with combination of FYM+$CaCO_3$.

In general, the extractability of Ni with DTPA and diacid from control. FYM and phosphate treated soils followed the same order of magnitudes (fig. 4a, 4b). The extraction of Zn and Cu, diacid was not able to extract Ni up to a detectable amount from soils where $CaCO_3$ is added either alone or in combination with FYM+$CaCO_3$.

DTPA and diacid to extract metals from control, FYM and phosphate treated soils followed the same order of magnitudes. But the diacid could not extract the metals up to analytical measurable limit (flame AAS) from soils where $CaCO_3$ is added either alone or in combination with FYM+$CaCO_3$. To have a clear picture of relative extractability of these two reagent under different treatments, mean values of extractable metals were computed separately for control, FYM and phosphate treated soils, and $CaCO_3$ and FYM+$CaCO_3$ treated soil as given in table 9a (6 months). The mean values of extractable metals were also computed taking all treatments together.

The mean values of extractable metals for extractant DTPA and diacid as given in table 9a,9b (after 6 and 12 months) shows that trend of extraction in increasing order is followed by DTPA and diacid with amendments, FYM and phosphate. But in the presence of amendments $CaCO_3$ and combination of amendments FYM+$CaCO_3$, while DTPA extracted measurable amount but amount extracted by diacid is not detectable.

DTPA extractable Zn, Cu, Cr, Ni, and Pb decreased significantly in the presence of lime ($CaCO_3$). The active $CaCO_3$ releases bicarbonates ions into the solution which decreases the solubility of Cr, Ni, and Pb, hence DTPA extractable metals decrease in the presence of lime. Besides, many other reasons attributed to the lime induced immobilization of metals include increases in negative charge, formation of strongly bound hydroxyl metal species; precipitation of metals as hydroxides, and sequestration due to enhanced microbial activity. Repeated application of lime at the rate of 2 to 10 t ha (every 2-5years) is necessary to maintain metal immobilization, has been suggest by Knox *et al*. 2001. Application of lime increases pH and thus decreases availability of metals.

Moreover, the neutral soil reaction (about pH 7.0) favours adsorption/precipitation of metal cations thus favouring removal of metals from solution (Kashem and singh, 2001). Redox potential is often considered an important factor although both increases and decreases in heavy metal solubility have been recorded following water logging and the onset of anaerobic soil condition (Charlatchka and Cambier, 2000; Chuan et al. 1996; Grybos et al., 2007). This is because a number of different processes occur following the onset of anaerobiosis and these often interact to effect metal solubility.

Other studies have noted an increase in DTPA extractable Ni in soil-sludge mixtures with time and this has been dscribed to dissolution of metal precipitates such as carbonates, hydroxides and phosphates through changes in pH or gas composition of the soil resulting from microbial activity, oxidation of metal sulphides to sulphates by autotrophic sulphur oxidizing bacteria; and microbial release of metals complexed with organic matter (Sander et al., 1987).

Effect of various treatments on drymatter yield of different species of Brassica was studed using pot culture experiments.

Data on *Brassica juneca* given in table 10 and fig. 5 indicates clearly that drymatter yield was maximum with amendment (SSP) over control, although the data with other amendments also indicates that amount is higher over control.

With *Brassica campestris* also drymatter yield was maximum with SSP and minmum with $CaCO_3$ values with other amendments are also higher over control as indicated in table 17 and fig.12.

With *Brassica carinata* data indicate that drymatter yield was maximum with amendment FYM and minimum with amendment $CaCO_3$ as indicated in table 24 and fig.19 with other amendments SSP and FYM+$CaCO_3$ yield was considerable higher over control.

When *Brassica napus* was tried using different amendments than as indicated that in table 31 and fig. 26 drymatter yield was maximum with FYM and minimum with $CaCO_3$ over control. Data indicate that with amendment SSP and combination of two amendments ($CaCO_3$+FYM) yield is considerable higher over control.

In the pot culture experiment *Brassica nigra* drymatter yield was observed with different amendments. Data in table 38 and fig.33 indicate that for this species drymatter yield is maximum with SSP and minimum with $CaCO_3$. Although with FYM and combination of $CaCO_3$+FYM, drymatter yield higher over control.

Comparative study of drymatter yield by different *Brassica species* in the presence of different amendments as indicated in table 45 shows that it is maximum in *Brassica carinata* followed *by Brassica napus, Brassica nigra, Brassica juneca* and *Brassica campestris.*

Hence drymatter yield in different *Brassica species* is in the following order.

B. carinata > *B. napus* > *B. nigra* > *B. juneca* > *B. campestris.*

Effect of various treatments of amendments and added metals on heavy metals concentration in different *species of Brassica* was studied and following results was obtained and at the time of flowering.

For Zn as data showen in table 11 and fig. 6 indicates that concentration of Zn is maximum with combination of $CaCO_3$+FYM and mimimum with $CaCO_3$. In case of Cu concentration was maximum with FYM while minimum with $CaCO_3$ (table 12 and fig.7).

For Ni concentration is maximum with FYM and minimum with SSP, when *Brassica juncea* was studied (table 13 and fig.8).

When amendments were added to the soil, metal concentration in plant was reduced due to immobilization of metal in soil by amendments.

For *Brassica campestris* as indicated by data in table 18 and fig.13 the concentration of Zn is maximum with FYM and minimum with $CaCO_3$, For Cu concentration was maximum with FYM but unlike Zn it is minimum with SSP (table 19 and fig.14).

For Ni concentration is maximum with FYM like Zn and Cu but minimum with SSP like Cu (table 20 and fig.15).

For *Brassica carinata* as indicated by data in table 25 and fig.20 that concentration of Zn is maximum with FYM and minimum with SSP.,

For Cu same trend was observed that is concentration Cu is maximum with FYM and minimum with SSP (table 26 and fig.21), For Ni concentration is maximum with FYM like Zn and Cu but minimum with $CaCO_3$ unlike Zn and Cu (table 26, 27).

For *Brassica napus* as indicated by data in table 32 and fig.27 the concentration of Zn was maximum with $CaCO_3$ and minimum with SSP, for Cu concentration is maximum with FYM and minimum with combination of $CaCO_3$+FYM as indicated in table 33 and fig.28.

For concentration of Ni it is maximum with FYM like Cu and minimum with $CaCO_3$ as indicated in table 34 and fig. 29.

When *Brassica nigra* was studied then it was observed that concentration of Zn is maximum with SSP and minimum with $CaCO_3$ as indicated in table 39 and fig.34, for Cu concentration is maximum with FYM and minimum with SSP as indicated by data given in table 40 and fig.35.

For Ni concentration was maximum with FYM like Cu and minimum with $CaCO_3$ unlike Cu as indicated in table 41 and fig.36.

When amendments were added to the soil, metal concentration in plant was reduced due to immobilization of metal in soil by amendments.

Camparative study of the concentration of Zn by different *species of Brassica* using different amendments is given below (table 46).

With amendment FYM concentration is minimum *in Brassica nigra* while maximum in *Brassica campestris,* with $CaCO_3$ concentration is minimum in *Brassica nigra* followed by continues increase in *Brassica juneca, Brassica carinata, Brassica campestris* and *Brassica napus.*

With amendment SSP concentration in ascending order *is Brassica nigra* (minimum), *B. juneca, B. napus, B. carinata* and *B. campestris* (maximum).

With combination of amendments $CaCO_3$+FYM ascending order of concentration of Zn is *B. nigra* (minimum*), B. juneca, B. napus, B. carinata* and *B.campestris* (maximum).

When concentration of Cu in different *Brassica species* using different amendments was studied then with each amendments same trend in ascending order was observed. That is *B. napus* (minimum), *B. carinata, B. nigra, B.campestris* and *B.juneca* (maximum) (table 47).

When Ni concentration in different *Brassica species* using different amendments is comparative discussion given below (table 48).

With amendment FYM, ascending order of concentration is *B. juneca* (minimum), *B. nigra, B. napus, B. campestris* and *B. carinata* showing very less difference between last three species.

With $CaCO_3$ ascending order is *B. juneca* (minimum), *B. nigra, B. carinata, B. napus* and *B. campestris* (maximum), With SSP ascending order is *B. Juneca* (minimum), *B. nigra, B. campestris, B. napus* and *B. carinata* (maximum).

With combination amendments $CaCO_3$+FYM ascending order of concentration of the Ni in different *Brassica species* is *B. juneca* (minimum), *B.nigra, B. campestris, B.carinata* (although very small difference in concentration was observed in *B. campestris* and *B. carinata*) and *B.napus* is maximum (table 48).

Effect of various treatments of amendments and added metals on metals uptake in different *Brassica species* was observed at the time of flowering and following results were obtained

Uptake of Zn metals in *Brassica juneca* was studied in the presence of different amendments data given in table 14 and fig.9 indicate that Zn uptake was maximum in the presence of amendment SSP and minimum in the presence of $CaCO_3$.

For Cu uptake in *Brassica juneca* unlike Zn. It is maximum in the presence of amendment FYM and minimum in the presence of amendment $CaCO_3$ as indicated by data given in table 15 and fig.10.

Uptake of Ni metal of *Brassica juneca* as given in table 16 and fig.11. It is maximum in the presence of FYM and minimum in the presence of $CaCO_3$

When amendments were added to the soil, metal uptake in plant was reduced due to immobilization of metal in soil by amendments.

When *Brassica campestris* was studied then metals uptake in the presence of different amendments is given below.

Uptake of Zn as indicated in the table 21 and fig.16. It is clear that uptake of Zn is maximum in the presence of amendment SSP and minimum in the presence of amendment $CaCO_3$. Ascending order of Zn is given below.

Maximum value is with $CaCO_3$ (569.16 μg pot^{-1}), FYM (698.53 μg pot^{-1}), combination of $CaCO_3$+FYM (709.23 μg pot^{-1}) and SSP (716.93 μg pot^{-1})

Uptake of metal Cu as indicated in table 22 and fig.17 uptake is maximum in the presence of FYM and minimum in the presence of SSP. Ascending order is SSP, combination of FYM+ $CaCO_3$, $CaCO_3$ and FYM.

Uptake of Ni by *Brassica campestris* is given in table 23 and fig.18. It is clear from the data that Ni uptake is maximum with FYM and minimum with amendment $CaCO_3$.

Metals uptake by *Brassica carinata* in the presence of different amendments was studied at the time of flowering

Data given in table 28 and fig. 23 about uptake of Zn indicate that it is maximum in the presence of FYM while minimum in the presence of amendment $CaCO_3$

Data on uptake of Cu by *Brassica carinata* in the presence of different amendments given in table 29 and fig.24 indicate that Cu uptake is maximum with FYM and minimum with SSP.

Ni uptake given in table 30 and fig.25 by *Brassica carinata* shows that Ni uptake is maximum with FYM and minimum with $CaCO_3$. Ascending order of uptake of Ni by *B.carinata* using different amendments is in the following order $CaCO_3$ (minimum), $CaCO_3$+FYM, SSP and FYM (minimum).

When *Brassica napus* was studied then following data were observed for uptake of metals using different amendment at the time of flowering.

Zn uptake as indicated by data in table 35 and fig.30 show that uptake of Zn is maximum with FYM and minimum with SSP.

Uptake of Cu by *B. napus* using different amendments given in table 36 and fig.31 indicate that uptake is maximum with FYM and minimum with combination of amendment $CaCO_3$+FYM.

Ni uptake by *B. napus* in the presence of different amendments is given in table 37 and fig.32 these data indicate that uptake of Ni is maximum with FYM and minimum with $CaCO_3$.

When amendments were added to the soil, metal uptake in plant was reduced due to immobilization of metal in soil by amendments.

The study of metals uptake by *Brassica nigra* at the time of flowering using different amendments is given below.

Uptake of Zn by *B.nigra* given in Table 42 and fig.37 indicate that it is maximum with SSP and minimum with $CaCO_3$.

Case of Cu uptake there is no much difference in the uptake amount using different amendments although values is maximum with FYM and minimum with the combination of two amendments $CaCO_3$+FYM (table 43 and fig. 38).

Ni uptake by *B. nigra* given in table 44 and fig.39 indicate that value is maximum with FYM while minimum with combination of two amendments $CaCO_3$+FYM like Cu.

Comparable study of Zn uptake by different *Brassica species* in the presence of different amendments as indicated in table 49 shows that in case of amendment FYM, SSP, and combination of $CaCO_3$+FYM maximum uptake was by *Brassica carinata*. While in the presence of amendment $CaCO_3$ maximum uptake was *Brassica napus*. Ascending order of Zn uptake with amendments FYM, SSP and combination of two amendments $CaCO_3$+FYM is identical given as *B. juneca, B. campestris, B. nigra,B. napus* and *B. carinata*. Only in case of amendment $CaCO_3$ Zn uptake by *B. napus* is more than *B. carinata* remaining order being identical for *B. juneca, B. campestris* and *B. nigra*.

Comparable study of Cu uptake as given in table 50 shows that ascending order for *B. campestris, B. napus*, and *B.carinata* is identical with all amendments given as *B. campestris, B. napus* and *B. carinata*. Remaining two species show a little change for example with SSP *B. juneca* uptake Cu is more than *B. nigra*. Same trend is with combination of two amendments for *B. juneca* and *B. nigra*.

Comparable study of Ni uptake by three species *i.e. B. juneca, B. campestris and B. nigra* follows the same trend with all amendments and ascending order being *B. juneca, B. campestris* and *B. nigra* as given in table 51 remaining two species Ni uptake with FYM is more in *B. carinata* than *B. napus* but with $CaCO_3$ order is reverse *i.e*. Ni uptake by *B. napus* is more than *B. carinata*. Same order is observed with combination of two amendments while with SSP trend is identical as with amendment FYM.