RESULTS

4.1 WATER CULTURE

4.1.1 Germination Percentage

The germination percentage of *B. juncea* seeds cultured in Petri plates is given in Table 4.1 and Fig 4.1. In single metal treatments, a decrease in germination percentage occurred with increase in the concentration of heavy metals in the medium. Maximum reduction (45%) was caused by Cr(VI) at 100 mg l\(^{-1}\), whereas Mn and Zn caused least inhibition (Table 4.2). Even at the highest concentration of Mn and Zn at 100 mg l\(^{-1}\), the percentage germination was observed to be 85% and 84% respectively. The order of inhibition by different heavy metals was Cr>Ni>Co>Cu>Mn>Zn. Two-way ANOVA for germination percentage of *B. juncea* seeds grown in binary combination (Table 4.3) shows statistically significant differences among mean germination percentage values in treatments with both the metals. The interaction between Cr and Mn was also found to be significant.

Multiple regressions without interaction (Table 4.4) reveal that there is significant correlation between germination percentage and the metals in combination for all the binary combinations, β-regression coefficients due to both the metals (X\(_1\) and X\(_2\)) were negative in all the binary treatments. However, better correlations were obtained when interaction models were used (Table 4.5). In all the binary treatments, the β-regression coefficients for both the metals were negative showing that the increase in concentration of either of the heavy metals, eliminating the effect of the second metal, decreased the percentage germination of *B. juncea* seeds. In most of the binary combinations, the interactive effect of both the metal caused decrease in germination percentage, thereby showing synergism between them. At 25 mg l\(^{-1}\) of both the metals in binary combinations (Cr+Ni), (Cr+Co) and (Cr+Cu), the percentage germination was observed to be positive *i.e.*, both the metal ions acted antagonistic to each other. The comparison of β-regression coefficients of the heavy metals in binary combinations revealed that Cr(VI) in combination with other metals was more effective in decreasing the germination percentage of the *B. juncea* seeds. Mn in combination with other heavy
metals (Ni, Co and Cu) exerted less negative effect. In binary combinations of Ni, the deleterious effect of Ni was more than the other metals (Co, Cu and Zn). Co in binary combinations with Cu and Zn, was more effective than Cu but less effective than Zn. In (Cu+Zn), the negative effective of Cu was greater than Zn.

Path analysis (Table 4.6) shows that the germination percentage decreased due to the direct effect of both the metals in all the binary combinations of Mn and Zn except for the combination (Mn+Zn). The total indirect effect of metals were insignificant, however the indirect effect of the interaction between the metals has negative effect on germination. Maximum negative indirect effect was by (Zn+Ni) and (Zn+Cu), both being -0.74. The interaction between (Mn+Co) caused greatest direct negative effect.

4.1.2 Root Length

The IC\textsubscript{50} values calculated on the basis of root length inhibition are given in Table 4.7. Cr was found to be the most toxic metal. Root growth was drastically reduced to a minimum value of 0.14 cm at 100 mg l\textsuperscript{-1} of Cr. The order of reduction of root length of \textit{B. juncea} seedlings by heavy metals was observed to be, Cr>Cu>Ni>Co>Mn>Zn. The individual and combined effects of heavy metals on root length of \textit{B. juncea} seedlings are given in Table 4.8 and Fig. 4.2. It was observed that in single metal treatments, as the concentration of heavy metals increased from 25 mg l\textsuperscript{-1} to 100 mg l\textsuperscript{-1} there was a corresponding decrease in the root length. Cr and Cu at 100 mg l\textsuperscript{-1} caused maximum inhibition, by 98.6% and 97.7% respectively as compared to the control.

In binary combinations, it was observed that Zn and Mn in combination with other metal decreased their inhibitory effects on root length (Table 4.9). At (Zn+Cr) 25 mg l\textsuperscript{-1}, (Zn+Ni) 25 mg l\textsuperscript{-1}, (Zn+Co) 25 mg l\textsuperscript{-1} and (Zn+Cu) 25 mg l\textsuperscript{-1}, the increase in root length was observed to be 303.0%, 78.2%, 14.4% and 270.6% respectively as compared to the individual applications of Cr, Ni, Co and Cu at the same concentration. Similarly, addition of 25 mg l\textsuperscript{-1} Mn to Cr at a concentration of 100 mg l\textsuperscript{-1} increased the root length by 2057.1% as compared to the single metal treatment of Cr. However, increasing the concentrations of Cr, Co, Cu and Ni in combination with Zn and Mn, inhibited the root
length more as compared to the individual applications of Zn and Mn. At 25 mg l⁻¹ Zn, addition of 25 mg l⁻¹ of Cr, Co, Cu and Ni, decreased the root length by 39.8%, 21.1%, 31.7% and 31.7% respectively. In the combinations of Mn with other metals, increasing doses of Cr, Co, Cu and Ni, also decreased the root growth as compared to Mn applied alone. Further, it was observed that in (Cr+Co), (Cr+Cu) (Ni+Co), (Ni+Cu) and (Co+Cu), both the metals in combination further decreased the root length of *B. juncea* seedlings. However, 25 mg l⁻¹ of Ni was found to be effective in increasing the root length as compared to the *B. juncea* seedlings individually treated with 25, 50 and 100 mg l⁻¹ of Cu. Similarly, addition of increasing concentrations of Co to Cu 50 mg l⁻¹ and Cu 100 mg l⁻¹ Cu increased the root length as compared to the application of Cu 50 mg l⁻¹ and Cu 100 mg l⁻¹ alone. Two-way ANOVA for root length of *B. juncea* seedlings grown in binary combination of heavy metals (Table 4.10) showed statistically significant differences among mean root lengths in combination with other heavy metals. All the binary interactions were also found to be significant.

Multiple regression analysis for root length as a function of binary combinations (Table 4.11) revealed that correlations between root length and the metals in combinations, for all the binary treatments were statistically significant. Better correlations were obtained using multiple regression interaction model (Table 4.12). The negative $\beta$-regression coefficients of all the metals showed decrease in root length under the influence of all the six metals. However, the interactive effects of these metals in combination were observed to be positive on root growth, thereby showing antagonistic interactions among the metal ions. The comparison of $\beta$-regression coefficients of all the metal showed that the toxicity of Cr is more than that of other metals in combination. Ni in combination with Co, Mn and Zn exerted greater negative effects except in (Ni+Cu) where Cu is more toxic than Ni. Similarly, negative value of $\beta$-regression coefficient of Co in (Co+Zn) was greater as compared to (Co+Cu). Cu showed more inhibitory effect on root growth than the other metals in binary combinations (Co+Cu) and (Cu+Zn). Both Mn and Zn were least toxic as indicated by their low negative values of $\beta$-regression coefficients.

Path analysis (Table 4.19) shows that in all the binary combinations of Mn and Zn with other heavy metals, the direct effect of both the metal ions decreased the root
length, whereas the direct effect of the interaction between them caused a positive effect on the shoot length of the seedlings, maximum by (Zn+Co), being 0.73. The total indirect effect of all the metals was positive but the individual effect of interaction of all the binary combination was negative.

4.1.3 Shoot Length

The relative change in shoot lengths of *B. juncea* seedlings cultured in Petri plates containing different concentrations of heavy metals (single or in binary combinations) is given in Table 4.14 and Fig. 4.3. It was observed that increasing concentrations of heavy metals whether applied singly or in binary combinations decreased the shoot growth of the seedlings. Cu caused maximum reduction in shoot length. At 100 mg l\(^{-1}\), the reduction in shoot length of different heavy metals was observed to be, Cu>Cr>Ni>Co>Mn>Zn.

Metals in binary combinations also exerted overall negative influence by decreasing the shoot length of the seedlings as compared to the controls. Cu and Cr in combination with other metals (Mn, Ni, Co, Cu and Zn) further reduced the shoot lengths of the seedlings as compared to the seedlings grown in single treatments of these metals. On addition of Cr (25 mg l\(^{-1}\)) to Mn, Ni, Co and Zn, all at 25 mg l\(^{-1}\), the shoot growth was decreased by 19.6%, 37.4%, 48.7% and 14.4% respectively as compared to the application of these metals individually. However, addition of increasing doses of Zn and Mn to Cr treatments caused increase in shoot growth. Supplementation of Mn to Cr (100 mg l\(^{-1}\)) containing media increased the shoot length of the seedlings. Similarly increasing doses of Zn increased the shoot growth at all the concentration of Cr (Table 4.15). Similar trends were observed in (Ni+Zn), (Co+Zn) and (Cu+Zn), where the presence of Zn compensates for the toxic effects of Ni, Co and Cu. Two-way ANOVA (Table 4.16) for shoot length of *B. juncea* seedlings grown in binary combinations of heavy metals shows statistically significant differences among mean shoot length values in treatment with both the metals. All the binary interactions were found to be significant.

Multiple regression analysis (Table 4.17) for shoot length reveals significant correlations with metals in combination for all the binary treatments. Except for Zn in
(Cr+Zn), negative β-regression coefficients of all the metals showed deleterious effects of heavy metals on the shoot growth with their increasing concentrations in the medium. Multiple regression with interaction (Table 4.18) further revealed that although the individual effect of each heavy metal on shoot length is inhibitory in nature, their interactive effects on shoots growth are positive, thereby showing antagonistic interactions between the two metal ions in binary combinations that mutually decrease their toxic effects on shoot growth. A comparison of β-regression coefficients of heavy metals showed that all the metals exerted negative influence on the shoot growth at varying degrees. The inhibitory effects of Cr and Cu in their binary combinations are more as compared to other metals. Ni in (Mn+Ni) and (Ni+Zn) showed more deleterious effects on shoot growth as compared to Mn and Zn respectively. Similarly, in its binary combinations, the effect of Co in decreasing the shoot length was greater than Mn, Ni and Zn. It was further observed that the overall effect of Mn and Zn in contributing the reduction in shoot growth was less as compared to Cr, Co, Cu, and Ni. However in binary combinations of Mn and Zn, Mn appeared to be more toxic.

Path analysis (Table 4.19) shows that in all the binary combinations of Mn and Zn with other heavy metals, the direct effect of both the metal ions decreased the shoot length, except for Zn in (Zn+Cr), (Zn+Co) and (Zn+Cu), whereas the direct effect of the interaction between them caused a positive effect on the shoot length of the seedlings, maximum by (Zn+Co), being 0.83. The total indirect effect of all the metals was positive but the individual effect of interaction of all the binary combination was negative.

4.1.4 Dry Weight

The effect of heavy metals either single or in binary combinations on the dry weight of *B. juncea* seedlings is given in Table 4.20 and Fig. 4.4. It was observed that with increase in concentration of heavy metals, there was a progressive decrease in the dry weight of the seedlings, except for Zn and Mn at 25 mg l\(^{-1}\), that have slightly stimulatory effect on the dry weight. Cr caused maximum decrease in the dry weight of the seedlings. At 100 mg l\(^{-1}\), the reduction in dry weight was observed to be 39.8% as
compared to the control. The order of inhibitory effect of different heavy metals on the
dry weight of *B. juncea* seedlings was observed to be, Cr>Co>Ni ~ Cu>Mn>Zn.

Both the metals in binary combinations decreased the dry weight of the
seedlings as compared to the control. Cr in combination with other metals decreased the
dry weight of the seedlings at all the test concentrations. On addition of even a low
concentration of Cr (25 mg l\(^{-1}\)) to Mn, Ni, Co, Cu and Zn containing solutions, there
was a significant decrease in dry weight of seedlings by 20.4%, 16.9%, 15.9%, 12.9%
and 27.9% respectively, as compared to the seedlings grown in their single metal
treatments. Similar trends were also observed in binary combinations of Ni, Co and Cu.
It was further observed that the presence of Zn in binary combinations of Cr, Ni, Co and
Cu, increased the dry weight as compared to the dry weight of the seedlings grown in
individual treatments of these metals. Increasing concentrations of Zn to all the test
concentrations of Cr and most of the test concentrations of Ni, Co and Cu, increased the
dry weight of the seedlings (Table 4.21). Similar behavior was also shown by Mn in
combination with Cr, where increasing doses of Mn (25, 50 and 100 mg l\(^{-1}\)), increased
the shoot lengths of the seedlings. Two-way ANOVA for dry weight of *B. juncea*
seedling grown in binary combinations (Table 4.22) shows statistically significantly
differences among mean dry weight values in treatments with both the metals.

Multiple regression analysis (Table 4.23) revealed significant correlations
among different binary combinations and the dry weight of the seedlings. \(\beta\)-regression
coefficients of both the metals in all the binary combinations were negative implying
that with increase in the concentration of either of the two metals, there was a
corresponding decrease in the dry weight. Multiple regression interaction model (Table
4.24) further revealed antagonistic interactions between the two metals in most of the
binary combinations, thereby showing positive interactive effects of the two metal ions
on the dry weight of the seedlings. In (Cr+Ni) and (Mn+Zn), the interactions observed
were synergistic in nature. A comparison of \(\beta\)-regression coefficients of all the metals in
binary combinations showed that Cr in its binary combinations, exerted more negative
effect as compared to coexisting metal ions. Similar behaviour was also shown by Ni.
Co showed greater negative effect than Cu and Zn in its respective binary combinations.
In (Cu+Zn), Cu proved to be more toxic. However, in all the binary combinations of Mn
and Zn, both the metals showed least negative effect on the dry weight of the seedlings as compared to other metals.

Path analysis (Table 4.25) shows that in all the binary combinations of Mn and Zn with other heavy metals, the direct effect of both the metal ions decreased the dry weight, except for Zn in (Zn+Cr). The interaction between metal ions in all the binary combinations caused a positive effect on the dry weight of the seedlings, except for (Mn+Zn). The total indirect effect of both the metals in binary combination was positive except for Mn and Zn in (Mn+Zn), whereas the indirect effect of interactions was observed to be negative, maximum being 0.81 by (Mn+Cr).

4.1.5 Heavy metal uptake

The heavy metal uptake in *B. juncea* seedlings grown in different binary combinations are given in Table 4.26 and Fig.4.5. It was observed that metal uptake in *B. juncea* increased with increasing concentrations in the medium. *B. juncea* seedlings showed maximum uptake of Zn and Mn, being 0.531 mg g⁻¹ dw and 0.445 mg g⁻¹ dw respectively at 100 mg l⁻¹ of the metal treatment, whereas Ni was accumulated the least (0.135 mg g⁻¹ dw). The order of metal uptake in the seedlings was observed to be, Zn>Mn>Cu>Co>Cr>Ni.

In order to understand the mechanism of uptake of heavy metals, double reciprocal plots were drawn between reciprocals of metal accumulation in the seedlings and reciprocal concentrations of metals in the medium (Table 4.27). The correlation coefficients of linear regressions of Mn and Co were significant at 0.05% level of significance. This reciprocal relationship between the variables follows the Michaelis-Menton equation. It is therefore inferred that the uptake of Mn and Co is carrier mediated.

Regarding metal uptake in binary combinations, it was observed that in all the binary combinations of Cr with other metals, both the metal ions mutually inhibited the uptake of each other (Table 4.28). At Cr (25 mg l⁻¹), with the addition of increasing doses of Mn (25 mg l⁻¹ and 100 mg l⁻¹) there was inhibition of Cr uptake by 19%, 27% and 40% respectively. Similarly, at Mn, 25 mg l⁻¹, increasing doses of Cr (25 mg l⁻¹ and 100 mg l⁻¹) decreased the Mn uptake by 26%, 32.1% and 35% respectively. Similar
trend was observed in the binary combinations of Cr with other heavy metal such as (Cr+Ni), (Cr+Co), (Cr+Cu) and (Cr+Zn). Two-way ANOVA (Table 4.29) reveals that there are statistically significant differences among the mean value content of all the metals in plants with respect to their application in binary combinations, except for Ni uptake in (Ni+Co) and Co uptake in (Co+Cu). Cr significantly affected the uptake of Mn, Ni, Cu and Zn. In all the binary combinations of Mn, both the metal ions significantly affected the uptake of each other, except for Ni uptake in (Mn+Ni). Ni significantly affected the uptake of Co, Cu and Zn. In (Co+Cu), (Co+Zn) and (Cu+Zn), both the ions significantly affected the uptake of each other. The interaction between (Cr+Mn), (Cr+Co), (Cr+Zn), (Cu+Mn), (Ni+Co), (Ni+Cu), (Cu+Co), (Co+Zn) and (Cu+Zn) was also found to be significant.

Multiple regression analysis (Table 4.30) revealed significant correlations between all the binary combinations and the metal uptake except for Ni uptake in (Mn+Ni) and (Ni+Zn), Co uptake in (Mn+Co) and Cu uptake in (Ni+Cu). However, better correlations were obtained by multiple regression interaction model (Table 4.31), where significant correlations between Co and Cu uptake in (Mn+Co) and (Mn+Cu) were also obtained. It was observed that in most of the binary combinations, mixed interactions among coexisting metal ion occurred in which both the metal ions inhibited the uptake of each other. In (Cr+Cu) both the metal ions facilitated the uptake of each other, but their interactive effects on Cr and Co uptake were negative, thereby showing antagonistic interaction between Cr and Co. Similar trend was also observed in (Ni+Co) and (Ni+Zn), where Ni showed antagonistic interactions with Co and Zn. In (Mn+Zn), Mn facilitated the uptake of Zn, but the antagonistic interaction between Mn and Zn, greatly decreased the Zn uptake. Similarly, Mn facilitated the uptake of Co and Cu but the interactive effect of Mn with Cu and Co decreased the uptake of both Cu and Co. In (Co+Cu), Co facilitated the uptake of Cu, but the antagonistic interaction between Co and Cu, inhibited the Cu uptake. A comparison of β-regression coefficients of all the metals showed that maximum inhibitory effect was caused by Cu on Ni in (Ni+Cu) combination.

Path analysis (Table 4.32) shows that in binary combinations of Mn, the uptake of all the metals increased due to their own direct effect. There was a decrease in the
uptake of Mn and Cr due to direct effect of the other metal in combination, whereas the uptake of Co and Cu increased due to the direct effect of Mn. Interaction between Mn-Cr, Mn-Co and Mn-Cu mutually decreased the uptake of each other. The binary combinations of Zn also followed the same trend of increased uptake of all the metals due to their own direct effect. In the binary combination (Zn+Ni), both the metal ions mutually facilitated the uptake of each other through their direct effect, whereas Zn uptake was inhibited by the direct effect of Cr, Co and Cu, in turn, the uptake of Cr, Co and Cu was also decreased due to direct effect of the Zn. Also, interactions between Zn and other heavy metals mutually decreased the uptake of each other through direct effect. All the metals in binary combinations of Mn mutually decreased the uptake of each other through indirect effect, maximum negative indirect effect (-0.92) was by Mn on Cu uptake in (Mn+Cu). However, the indirect effect of the interaction occurring between them caused positive effect on the metal uptake except in case of Mn uptake in (Mn+Ni).
4.2 SAND CULTURE

4.2.1 Germination percentage

The germination percentage of *B. juncea* seeds grown in sand culture is given in Table 4.33 and Fig. 4.6. In single metal treatments, a corresponding decrease in germination percentage occurred with increase in the concentration of heavy metals in the medium. Cr(VI) at 100 mg kg\(^{-1}\) caused maximum reduction (38%) followed by Co (65%), whereas, Mn and Zn caused least inhibition (84% and 82% respectively) even at highest concentration of 100 mg kg\(^{-1}\). The order of inhibition by different heavy metals was Cr>Co>Cu>Ni>Zn>Mn. Percentage change in the germination percentage of the seedlings was given in Table 4.34. Two-way ANOVA for germination percentage of *B. juncea* seeds grown in binary combinations (Table 4.35) shows statistically significant differences among germination percentage values with both the metals in the binary treatments (Cr+Mn), (Cr+Co), (Cr+Cu), (Cr+Zn), (Cu+Mn) and (Ni+Zn). The interactions between (Cr+Zn), (Cu+Mn), (Cu+Ni), (Ni+Zn) and (Co+Cu) were also found to be significant.

Multiple regression analysis, with or without interaction (Tables 4.36-4.37) revealed significant correlation between germination percentage and the metals in binary combinations. In all the binary treatments, (except for treatments having Mn and Zn), \(\beta\)-regression coefficients for both the metal were negative showing that both the metal ions independently decreased the germination percentage. However, the interactive effects of binary treatments were positive thereby, showing antagonistic interaction occurring between the metal ions that mutually ameliorate the toxicity of each other. The comparison of \(\beta\)-regression coefficients of the heavy metals revealed that in all its binary combinations, Cr emerged to be more toxic than its coexisting metal ions, whereas Mn and Zn exerted less negative effect than their counterparts (Ni, Co, Cu and Cr). In the binary combinations (Ni+Zn) and (Ni+Cu), Ni showed more deleterious effect than Zn and Cu, whereas in (Ni+Co), the toxicity of Ni was suppressed by Co. Co was more effective in decreasing the germination than its coexisting ions in all its binary combinations, however, Cr suppressed its toxicity in (Co+Cr). Cu also appeared more toxic in (Mn+Cu) and (Cu+Zn), but in the presence of Cr, Ni and Co, its toxic effect was suppressed.
4.2.2 Root length

The individual and combined effects of heavy metals on root length of *B. juncea* seedlings are given in Table 4.38 and Fig. 4.7. There was a corresponding decrease in the root length as the metal concentrations increased in medium. However, Zn and Mn at low concentrations (25 and 50 mg kg⁻¹) showed stimulatory effects as root length increased by 9.8% and 11.4% at 25 mg kg⁻¹ of Mn of Zn respectively. Cr caused maximum retardation (1.58 cm) followed by Ni (2.63 cm) at 100 mg kg⁻¹. The observed reduction of root growth by heavy metals yielded a toxicity series, Cr>Ni>Cu>Co>Zn>Mn.

It was observed that Zn and Mn in binary combinations with Cr decreased its inhibitory effect. In (Mn+Cr) and (Cr+Zn), both metals at 25 mg kg⁻¹, the increase in root length was observed to be 22% and 30.3% respectively as compared to the individual applications of Cr alone (Table 4.39). Supplementation of Zn to the medium containing increasing concentrations of Ni, Co and Cu also followed the similar trend. However, additions of Cr, Co, Ni and Cu to Mn and Zn containing media inhibited the root length to a greater extent than the individual applications of Mn and Zn. Similarly, other binary combinations containing Ni, Co, Cu and Cr also reduced the root length of the seedlings as compared to the control, but at few effective concentrations, their effects were mutually suppressed and the root growth was increased as compared to their individual treatments. At the concentrations (Ni25+Co25), (Ni25+Cu25) and (Co25+Cu25) mg kg⁻¹, root length was increased by 14.3%, 4.0% and 3.5% as compared to the single metal treatments of Ni and Co respectively. Two-way ANOVA (Table 4.40) showed statistically significant differences among mean root lengths in treatments with both the metals, except for (Cr+Ni) and (Mn+Cu). All the binary interactions were also found to be significant, except for (Mn+Cu), (Mn+Ni) and (Mn+Co).

Multiple regression analyses (Tables 4.41-4.42) for root length as a function of metals in binary combinations revealed that for all the binary combinations, the correlations between root length and the metals in combinations were statistically significant. All the heavy metals, except for Mn and Zn in few combinations, exerted deleterious effects on root growth as indicated by their negative β-regression.
coefficients, however, the interactions among these metal ions showed positive effect on the root length either through antagonism or by mixed interaction where one metal ion suppressed the toxicity of the other. The comparison of β-regression coefficients of all the heavy metals revealed that in all its binary combinations, Cr caused maximum toxicity as compared to its coexisting ions. Ni also showed greater toxicity in (Ni+Co), (Ni+Cu) and (Mn+Ni) as indicated by its higher negative values of β-regression coefficients as compared to its coexisting ions. The β-regression coefficients of Co were also negative in all its binary combinations (Co+Cu), (Ni+Co), (Cr+Co) and (Co+Zn), but to a lesser extent than its coexisting ions (Ni, Cr, Cu and Zn). In (Mn+Co), it showed greater toxicity. In all the binary combinations, Mn exhibited positive β-regression coefficients except for the combination (Mn+Zn) whereas, the β-regression coefficients of Zn were negative in all its binary combinations.

4.2.3 Shoot length

The relative change in shoot lengths of *B. juncea* seedlings grown in sand cultures containing heavy metals in single metal treatments and in binary combinations is given in Table 4.43 and Fig. 4.8. Increasing concentrations of heavy metals whether applied singly (except for Mn and Zn) or in binary combinations significantly decreased the shoot length of the seedlings. Mn and Zn at the concentrations of 25 and 50 mg kg\(^{-1}\) stimulated the shoot growth by 6.2% and 2.2%, and 10.4% and 3.9% respectively. Cr caused maximum reduction in shoot length (1.78 cm) at 100 mg kg\(^{-1}\). On the basis of reduction in shoot length by different heavy metals, their order of toxicity was observed to be, Cr>Ni>Co>Cu>Mn>Zn.

Binary combinations of heavy metals also decreased the shoot length of the seedlings as compared to the control, except at few effective treatments where increase in the shoot length was observed as compared to the respective single metal treatments. At (Cr25+Ni25) mg kg\(^{-1}\), shoot length was decreased by 15.7% and 34.5% as compared to the individual treatments of Cr 25 mg kg\(^{-1}\) and Ni 25 mg kg\(^{-1}\) respectively. Similarly, at (Ni+Cu) 25 mg kg\(^{-1}\), shoot length was decreased by 20.8% and 27.7% as compared to individual treatments of Ni 25 mg kg\(^{-1}\) and Cu 25 mg kg\(^{-1}\) respectively (Table 4.44). On the other hand, at (Ni25+Co25) mg kg\(^{-1}\), shoot length was increased by 10.9% and
9.5% as compared to single treatments of Ni 25 mg kg\(^{-1}\) and Co 25 mg kg\(^{-1}\) respectively.

However, in all the binary combinations containing Zn, an increase in the shoot length was observed as compared to the individual applications of Ni, Co, Cu and Cr. In the binary combinations (Cr25+Zn25), (Ni25+Zn25), (Co25+Zn25) and (Cu25+Zn50), shoot length was increased by 17.4%, 35.6%, 10.5% and 15.4% respectively. Mn in its binary combinations with other heavy metals also followed the similar trend where Mn suppressed the toxicity of Ni, Co, Cu and Cr. Two-way ANOVA (Table 4.45) for shoot length of the seedlings showed statistically significant differences among mean shoot length values in treatments with both the metals. All the binary interactions were also found to be significant, except for (Cu+Mn), (Co+Zn) and (Cu+Zn).

Multiple regression analyses (Tables 4.46-4.47) showed significant correlations of seedling shoot length with metals in binary combinations. Although all the heavy metals showed negative \(\beta\)-regression coefficients in most of the binary combinations, the positive \(\beta\)-regression coefficients of interactions showed mutual amelioration of the toxicities of coexisting metal ions thereby exerting positive interactive effects on the shoot length of the seedlings. All the interactions occurring among metal ions were of mixed or of antagonistic nature. A comparison of \(\beta\)-regression coefficients of heavy metals showed that Cr caused more toxicity than its coexisting metal ions in all its binary combinations. In the binary combinations (Mn+Ni) and (Ni+Cu), Ni has higher value \(\beta\)-regression coefficient, but in (Cr+Ni), (Ni+Co) and (Ni+Zn) its toxicity was suppressed by its coexisting metal ions. Co also showed greater toxic effects in (Ni+Co) and (Mn+Co) but its effect was suppressed by Cr and Zn in (Cr+Co) and (Co+Zn). Among all the heavy metals, the overall effect of Mn on the shoot growth was mostly positive except for (Mn+Cu).

4.2.4 Dry weight

The effects of heavy metals on the dry weight of \(B.\) juncea seedlings are given in Table 4.48 and Fig. 4.9. With increasing concentrations of heavy metals in the medium, there was a progressive decrease in the dry weight of the seedlings, except for Zn at 25 mg kg\(^{-1}\) which showed a slight stimulatory effect on the dry weight. Maximum decrease in the dry weight (5.88 mg seedling\(^{-1}\)) was observed at the treatment of Cr 100 mg kg\(^{-1}\)
followed by Cu (7.75 mg/seedling). The inhibitory effect of different heavy metals on
the dry weight followed the order, Cr>Cu>Ni>Co>Zn>Mn. The heavy metals in
different binary combinations also decreased the dry weight as compared to the control;
maximum reduction (3.9 mg/seedling) was observed at (Cr100+Ni100) mg kg\(^{-1}\). In
binary combinations also, Cr caused maximum deleterious effects. On addition of even
low dose (25 mg kg\(^{-1}\)) of Cr to Mn, Ni, Co, Cu and Zn, the dry weight was reduced by
47.0%, 40.1%, 39.0%, 27.9% and 54.3% respectively, as compared to the seedlings
grown in the respective single metal treatments. However, presence of Zn and Mn in
binary combinations containing Cr, Ni, Co and Cu suppressed their toxicity and
increased the dry weight of seedlings as compared to the individual applications of
these metals. In the treatments of (Cr25+Mn25) mg kg\(^{-1}\) and (Cr25+Zn25) mg kg\(^{-1}\),
seedlings dry weight was increased by 75.3% and 86.5% respectively as compared to
individual application of Cr 25 mg kg\(^{-1}\) (Table 4.49). Similar behavior was also
observed where Mn and Zn were added to the medium containing varying
concentrations of Ni, Co and Cu. Two-way ANOVA (Table 4.50) showed statistically
significant differences among mean dry weight values in treatments with both the
metals. All the binary interactions were also significant.

Multiple regression analyses with and without interaction (Tables 4.51-4.52)
revealed significant correlations between the dry weight and the heavy metals in their
respective binary combinations. Although β-regression coefficients were negative for
all the heavy metals the interactive effects of all the binary combinations were positive
on the dry weight due to the antagonism between the coexisting metal ions which
mutually ameliorate their toxicity. A comparison of β-regression coefficients of all the
metals in binary combinations showed that Cr caused more negative effect as compared
to its coexisting ions. Ni also showed similar behavior in its combinations with Mn, Co,
Cu and Zn. Co and Cu were more toxic than Mn and Zn in their respective
combinations however, in (Co+Cu), Cu suppressed the toxicity of Co. Mn and Cu were
found to be the least toxic metals for seedling dry weight as indicated by their low
negative values of β-regression coefficients.
4.2.5 Heavy metal uptake

It was observed that *B. juncea* seedling showed maximum uptake of Zn and Mn, being 0.253 and 0.230 at 100 mg kg\(^{-1}\), whereas Cr was the metal least accumulated, being 0.103 mg g\(^{-1}\) dw (Table 4.53, Fig. 4.10). The order of metal uptake in the seedling was observed to be, Zn>Mn>Cu>Ni>Co>Cr.

In all the binary combinations both the metals mutually inhibited the uptake of each other except for few test concentrations where one metal facilitated the uptake of the other. Addition of increasing doses of Mn (25, 50 and 100 mg kg\(^{-1}\)) reduced the uptake of Cr by 24.2%, 54.5% and 51.5% as compared to the uptake of Cr at 25 mg kg\(^{-1}\) alone. In turn, addition of 25, 50 and 100 mg kg\(^{-1}\) of Cr also inhibited the uptake of Mn by 54.3%, 58.0% and 66.1% respectively as compared to Mn uptake at single treatment of 25 mg kg\(^{-1}\). Similar trend was also observed in (Cr+Zn). Two-way ANOVA (Table 4.54) reveals that there are statistically significant differences among the mean value content of both Cr and Mn, Cr and Zn, Co and Mn, and Cu and Mn in the seedlings with respect to their respective binary combinations in the medium. Cr uptake was significantly affected by Ni, Co and Zn. Zn uptake was significantly affected by Cr and Co. In (Zn+Cu), both the ions significantly affected the uptake of each other.

Multiple regression analysis (Table 4.55) revealed significant correlations between most of the binary combinations and the uptake of respective metals. However, better correlations were obtained by multiple regression interaction model (Table 4.56). In all the significant correlations obtained, the interactive effect of the interaction occurring among both the metals in the binary combinations caused decreased uptake of the respective metal ions. Most of the inhibitory effects caused were due to the antagonism between coexisting metal ions or mixed interaction where one ion suppressed the effect of the other that led to decreased uptake. However in (Cr+Ni), the uptake of Ni was increased due the mixed interaction where inhibitory effect of Cr was suppressed by Ni. Comparison of β-regression coefficients of all the metals showed that Mn showed highest negative value of β-regression coefficient in the combination (Cr+Mn).
4.3 POT CULTURE

4.3.1 Binary combinations

4.3.1.1 Seedling emergence

The effects of heavy metals applied, singly and in binary combinations, on the seedling emergence of *B. juncea* are presented in Table 4.57 and Fig. 4.11. It was observed that increasing concentrations of all the heavy metals in the soil medium significantly decreased the seedling emergence of *B. juncea* seeds. Maximum inhibitory effect was caused by Cr at 100 mg kg\(^{-1}\), where seedling emergence was only 6%. However, Mn and Zn, at concentration of 50 mg kg\(^{-1}\), significantly stimulated the seedling emergence by 16.5% and 24.9% respectively. The order of toxic effects of heavy metals on the seedling emergence was observed to be, Cr>Ni = Co>Mn>Zn.

The heavy metals applied in binary combinations also significantly decreased the seedling emergence as compared to the control. Cr, in all its binary combinations with other heavy metals, inhibited the seedling emergence, as compared to the single metal application. The presence of low dose of Cr at 50 mg kg\(^{-1}\) in (Cr50+Mn50), (Cr50+Ni50), (Cr50+Co50), (Cr50+Cu50) and (Cr50+Zn50), decreased the seedling emergence by 71.4%, 60%, 40%, 81.8% and 66.6% respectively as compared to the individual application of 50 mg kg\(^{-1}\) of these metals independently. Two-way ANOVA (Table 4.58) shows statistically significant differences among germination percentage values with Cr in all its binary combinations, Ni in (Mn+Ni) and (Ni+Zn), Co in (Co+Zn), and Cu in (Cu+Zn).

Multiple regression without interaction (Table 4.59) revealed significant correlation between seedling emergence and the metals in combination, for most of the binary treatments. β-regression coefficient of all the metals (except Mn and Zn in few combinations), were negative in all the binary combinations, thereby indicating their inhibitory effect on the seedling emergence. However, better correlations were obtained using multiple regression interaction modal (Table 4.60). The interaction was observed to be positive in all the combinations, due to the antagonistic interaction between both the metal ions, whereas, the negative interactive effect shown by (Mn+Co), (Ni+Zn), (Co+Zn), (Co+Zn) and (Cu+Zn) was due to the mixed interaction occurring between the
two ions of these combinations. In (Co+Cu), synergism was observed where both the metal ions, independently, as well as in interaction, inhibited the seedling emergence.

The comparison of $\beta$-regression coefficients of all the metals in binary combination, revealed that Cr is more effective in inhibiting the seedling emergence as compared to other heavy metals. In binary combinations of Ni and Co, both the metals proved to be more toxic than their counterparts, Cu, Zu and Mn. Among all the heavy metals, Zn and Mn emerged out to be least toxic metals as represented by their less negative values of $\beta$-regression coefficients.

4.3.1.2 Shoot length

The relative change in shoot lengths of B. juncea plants grown in soils containing different concentration of heavy metals is given in Table 4.61 and Fig. 4.12. It was observed that with increase in the concentration of heavy metals (Cr, Ni, Cu and Zn) in the medium, there was a progressive decrease in the shoot length of B. juncea plants. Cr caused maximum reduction in shoot length. At 100 mg kg$^{-1}$ concentration of Cr, the decrease in shoot length was found to be 92.9% as compared to the control. The order of reduction in shoot length by different heavy metals was observed to be, Cr>Cu>Ni>Zn>Co>Mn.

Heavy metals in binary combinations also exerted an overall negative effect on the shoot heights (except at few effective treatments). Cr in combination with other heavy metals (Mn, Ni, Co, Cu and Zn) at 50 mg kg$^{-1}$ further reduced the shoot length by 52.1%, 71.7%, 85.1%, 60.9% and 65.7% respectively, as compared to the individual application of these metals. However, addition of Mn and Zn to Cr treatments caused increase in the shoot growth. Supplementation of Mn at doses 50 and 100 mg kg$^{-1}$ to soil containing Cr 100 mg kg$^{-1}$ increased the shoot growth by 66.7% and 31.8% respectively. Similarly, increasing doses of Zn increased the shoot growth at all the test concentrations of Cr whereas, the presence of Ni, Co, Cu further enhanced the toxic effects of Cr on the shoot length.

Multiple regression analysis (Table 4.62) for shoot length of B. juncea plants showed significant correlations with metals in binary combinations. Multiple regression interaction model (Table 4.63) revealed better correlations among the variables. It was
observed that the interactive effects of heavy metals in most of the binary combinations were positive in nature. In (Mn+Zn) and (Co+Cu), increasing concentration of both the metals as well as their interactive effect decreased the shoot growth showing negative synergistic interaction between Mn-Zn and Co-Cu. In binary combinations (Cr+Co), (Cr+Ni) and (Cr+Cu), although both the metal ions independently decreased the shoot length, their interactive effect was positive thereby showing antagonism between the metal ions. In (Cr+Mn) and (Cr+Zn), positive interactive effect was caused due to the mixed interaction occurring between Cr-Zn and Cr-Mn, where Zn and Mn compensated the toxic effect of Cr. Also, Mn in binary combinations with Ni, Cu and Zn, alleviated the toxicity of its coexisting ions through mixed interactions and thus promoted the shoot growth of *B. juncea* plants. The comparison of β-regression coefficients of heavy metals showed that Cr and Cu, in binary combinations with other heavy metals, were more toxic. Ni in combination with Mn and Co had higher β regression coefficient. Co was more toxic than Mn in (Mn+Co) but less toxic than Zn in (Co+Zn) combination.

4.3.1.3 Biomass

**Root Biomass**

It was observed that with increasing concentrations of heavy metals, except for Zn and Mn, there was a progressive decrease in the root biomass (Table 4.64, Fig. 4.13). At Mn 50 mg kg\(^{-1}\) and Zn 50 mg kg\(^{-1}\), there was a significant increase in the biomass of roots by 18.2% and 22.7% respectively as compared to the control. Maximum reduction was caused by Cr. Increasing concentrations of Cr 50 mg kg\(^{-1}\) and 100 mg kg\(^{-1}\), decreased the root biomass by 36.2% and 50.7% respectively. However, supplementation of Mn and Zn increased the root biomass of *B. juncea* plants. The order of inhibiting effect of different heavy metals on the root biomass was observed to be, Cr>Ni>Cu>Co>Mn>Zn.

Multiple regression analysis (Table 4.65) showed significant correlations between several binary combinations and the root biomass. Multiple regression interaction model (Table 4.66) revealed positive interactive effects on root biomass due to the interactions occurring between the coexisting ions of binary combinations. It was observed that although Cr independently exerted negative effects on root biomass, it
showed positive interactive effect due to the occurrence of mixed and antagonistic interactions with its coexisting metal ions. In the binary combinations (Mn+Cr), (Cr+Co) and (Cr+Zn), Co, Mn and Zn alleviated toxicity of Cr and thus showed positive interactive effects. In (Cr+Ni) and (Cr+Cu), although increasing concentrations of both the metal ions exerted deleterious effects, the antagonism between Cr-Ni and Cr-Cu ions, also produced positive effect on the root biomass. Similar mixed interactions were also observed in (Mn+Co), (Mn+Ni), (Zn+Cu) and (Zn+Ni) where Mn and Zn counteracted the toxicity of Co, Ni, Cu, thus producing positive interactive effect on the root biomass of *B. juncea* plants. A comparison of β-regression coefficients of all the heavy metals showed that Cr and Ni, in binary combinations were more toxic than all their coexisting ions. Cu in the binary combinations of (Cu+Co) and (Cu+Zn) showed greater toxicity on root biomass than Co and Zn. Similarly, Co was more toxic than Zn in (Co+Zn). Among all the heavy metals, Mn and Zn showed no deleterious effect on the root biomass.

**Leaf biomass**

The effect of heavy metals (applied singly or in binary combinations) on the leaf biomass of *B. juncea* plants is given in Table 4.67 and Fig. 4.14. It was observed that the addition of heavy metals in the soil significantly decreased the leaf biomass as compared to the control. However, Zn and Mn exerted stimulatory effect on the leaf biomass. Addition of 50 mg kg$^{-1}$ of Mn and Zn, increased the leaf biomass by 15.5% and 2.1% respectively as compared to the control. At 100 mg kg$^{-1}$, Cu caused maximum reduction by decreasing the biomass by 55.5%. The order of reduction caused by heavy metals was observed to be Cu>Cr∼Ni>Co>Zn>Mn.

In binary combinations, there was a general decrease in leaf biomass except at few effective treatments. Zn and Mn were observed to increase the leaf biomass in all the binary combinations with other metals. Supplementation of 50 mg kg$^{-1}$ of Mn to Cr, Ni, Co, Cu and Zn, increased the leaf biomass by 23.2%, 45.7%, 53.6%, 36.2% and 20.2% respectively. Multiple regression analysis (Table 4.68) of biomass of leaves showed significant correlations between leaf biomass and few of binary combinations. However multiple regression interaction model (Table 4.69) showed better correlations
and further revealed the interactions occurring between the two metal ions of binary combinations. It was observed that in all the binary combinations of heavy metals, most of the heavy metals showed negative effect on the biomass of leaves, as indicated by their negative $\beta$-regression coefficients, but the interactive effect of heavy metals in combinations produced a positive effect. In (Cr+Ni), (Cr+Co) and (Cr+Cu), antagonistic interactions occurred between the metal ions causing a positive response, whereas in (Cr+Mn) and (Cr+Zn) mixed interactions were observed, where Mn and Zn compensated the toxicity of Cr thus, producing a positive effect on leaf biomass. Similarly in (Ni+Co), (Ni+Cu), (Mn+Ni) and (Co+Cu), both the metal ions independently caused reduction, however, the antagonistic interaction between them exerted a stimulatory effect on leaf biomass. Mixed interaction was also observed in binary combinations of (Mn+Co), (Mn+Cu) and (Cu+Zn) where Zn and Mn compensated the toxicity of Co and Cu, thereby causing positive effect on leaf biomass.

A comparison of $\beta$-regression coefficients showed that in all the binary combinations of Cr, Cr itself caused maximum toxicity than its coexisting ions. Ni in its binary combination with Mn, Co and Zn showed greater inhibitory effect on the leaf biomass. Similarly, Co in combination with Mn, Cu and Zn was more toxic than its counterparts in binary combinations. The positive $\beta$ regression coefficients of Mn and Zn indicated their positive effect on leaf biomass of B. juncea plants.

**Stem Biomass**

The effect of heavy metals on the stem biomass of B. juncea plants grown in single metal treatments and in binary combinations is presented in Table 4.70 and Fig. 4.15. It was observed that with increasing concentrations of heavy metals there was a progressive decrease in the biomass of stems of B. juncea plants, except for Zn and Mn. Cr caused maximum reduction in stem biomass as at 100 mg kg$^{-1}$ the stem biomass was reduced by 47.9% as compared to the control. Maximum biomass of stem was produced by Mn at the treatment of 50 mg kg$^{-1}$. The reduction of stem biomass due to heavy metals follows the order, Cr>Cu>Ni>Co>Mn>Zn.

The presence of Mn and Zn in binary combinations increased the stem biomass as compared to the biomass of plants grown in single metal treatment. Addition of 50
mg kg$^{-1}$ of Mn to Cr, Ni, Co, Cu and Zn, increased the stem biomass by 43.9%, 59.2%, 28.3%, 46.9% and 53.6% respectively. Similar stimulatory effect was also shown by Zn in binary combinations with other heavy metals.

Multiple regression analysis (Table 4.71) showed few significant correlations between stem biomass and the heavy metals in binary combinations, but multiple regression interaction model (Table 4.72) revealed better correlations and the interactions that occurred between the two metal ions in a binary combinations. Cr in combination with Mn and Zn showed mixed interaction, where Mn and Zn alleviated the toxic effect of Cr, thereby exerting stimulatory effect of the stem biomass. Mn in combination with Ni and Cu also showed similar interaction thus, compensating the toxicity of Ni and Cu. However, in (Mn+Zn), both the ions showed synergism thereby exerting positive effect on the stem biomass. In (Co+Cu), a strong correlation was observed where both the metal ions independently caused reduction but the antagonistic interaction between them generated a positive effect on the stem biomass. A comparison of $\beta$ regression coefficients showed that in the binary combination (Ni+Co), Ni caused maximum deleterious effect on the stem biomass of *B. juncea* plants.

**Fruit Biomass**

A progressive decrease in the fruit biomass with corresponding increase in the heavy metal concentration was observed (Table 4.73, Fig. 4.16). However Zn and Mn (applied singly) did not show any deleterious effect on the fruit biomass. At 100 mg kg$^{-1}$ Cr caused maximum reduction by 55.6% as compared to the control. Whereas addition of 50 mg kg$^{-1}$ of Mn, Ni, Co, Cu and Zn increased the fruit biomass by 41.3%, 18%, 38.4%, 16.3% and 52.3% respectively. The order of inhibitory effect of heavy metals on fruit biomass was observed to be, Cr>Co>Cu>Ni>Mn>Zn.

Supplementation of Mn and Zn to other heavy metals also increased the fruit biomass of *B. juncea* plants. Addition of 50 mg kg$^{-1}$ of Mn to Ni and Cu stimulated the production of fruit biomass by 20.6% and 15.2% respectively as compared to the fruit biomass of plants grown in individual applications of those metals. Similar effect of Zn was also observed in its binary combinations with other heavy metals.

Multiple regression analyses (with or without interaction) of fruit biomass as a function of binary combinations (Tables 4.74-4.75) revealed significant correlations
between all the binary combinations of Cr and the fruit biomass. In (Cr+Ni), (Cr+Co), (Cr+Cu) and (Ni+Co), increasing concentrations of both the metals caused reduction in fruit biomass but their antagonistic interaction produced an overall positive effect on the fruit biomass. Mn and Zn compensated the toxicity of Cr and Co in (Cr+Mn) and (Co+Zn) through a mixed interaction, thus generating a positive effect on fruit biomass. However in (Cr+Zn) and (Ni+Zn), Zn could not alleviate the toxicity of Cr and Ni, thereby showing synergism with Ni and thus, mixed interaction caused negative effect on fruit biomass. Among all the heavy metals, Cr was found to exert maximum deleterious effect on fruit biomass as indicated by its high negative values of $\beta$-regression coefficients in all its binary combinations.

**Total Biomass**

The presence of Zn in the soil medium produced an overall positive effect on the total biomass of the plants (Table 4.76, Fig. 4.17). In the presence of Zn (50 mg kg$^{-1}$), the total biomass of plants was raised to 15.11 g. Mn also exerted stimulatory effect on the biomass of the plant. All other heavy metals (Ni, Co, Cu and Cr) generated deleterious effects in terms of reduction in the total biomass. Cr caused maximum reduction of 100 mg kg$^{-1}$ (6.74 g plant$^{-1}$). However addition of Mn, Ni, Co, Cu and Zn, to Cr treatments, significantly raised the plant biomass. Maximum increase was caused by Zn and Mn. Supplementation of Mn and Zn to the treatments of Ni, Co and Cu also increased the total biomass of plants as compared to the plants grown in single application of these metals. Maximum plant biomass was obtained by the application of binary treatment (Zn+Mn).

Multiple regression analyses (Tables 4.77-4.78) revealed significant correlation between several binary combinations and the total biomass of the plants. All the three types of metal interactions occurred in the binary combinations of heavy metals that exerted an overall positive effect on the total biomass of plants. Cr in combination with Ni, Co and Cu showed antagonism with these metal ions. Although increasing concentrations of Ni, Co and Cu decreased the total biomass, the antagonism between Cr-Ni, Co-Cr and Cr-Cu mutually alleviated their toxicity causing a positive effect on the plant biomass. In (Cr+Mn) and (Cr+Zn), Mn and Zn compensated the toxicity of Cr
through mixed interaction in which Cr showed synergism with Mn and Zn, thus promoting the plant biomass. Mn in combination with Ni, Co and Cu also reduced the toxicity of Ni, Co and Cu through mixed interaction, thereby causing a positive effect. In (Mn+Zn) both the ions showed synergism that caused a stimulatory effect on the plant biomass. In binary combination of Ni, (Ni+Co), (Ni+Cu), (Ni+Zn) and (Co+Cu), the antagonistic interaction between the ions caused a positive influence on the plant biomass by mutually decreasing the toxicity of each other. A comparison of $\beta$ regression coefficients revealed Cr as the most toxic element (having highest negative value) that caused maximum deleterious effect in all its binary combinations with other heavy metals.

3.1.4 Heavy metal uptake

Roots

Heavy metal uptake in the roots of *B. juncea* plants grown in different binary combinations of heavy metals in given in Table 4.79 and Fig. 4.18. With increasing concentrations of heavy metals in the soil, there was corresponding increase in the uptake of heavy metals in the roots. The roots of *B. juncea* showed maximum uptake of Mn and Zn. At 100 mg kg$^{-1}$, uptake of Mn and Zn in roots was 0.105 mg g$^{-1}$dw and 0.206 mg g$^{-1}$dw respectively, whereas Cu was the least accumulated metal, being 0.023 mg g$^{-1}$dw. The order of metal uptake in the roots of *B. juncea* plants was observed to be, Zn>Mn>Co>Cr>Ni>Cu.

Metal uptake analysis of roots of *B. juncea* grown in binary metal combinations revealed that the uptake of metal ion was inhibited by the presence of coexisting ions, except in few treatments where one ion facilitated the uptake of other ion at particular concentrations. At Cr (50 mg kg$^{-1}$) increasing doses of Mn (50 mg kg$^{-1}$ and 100 mg kg$^{-1}$) decreased the uptake of Cr by 25.3% and 42.2% respectively. Similarly, increasing concentration of Cr inhibited the uptake of Mn by 56.6% and 74.5% respectively. Similar trend was observed in the binary combination (Cr+Zn) where both the ions mutually inhibited the uptake of each other. Two-way ANOVA (Table 4.80) reveals that there are statistically significant differences among the mean value content of all the metals in the root with respect to their respective binary treatments in the soil.
except for Ni uptake in (Cr+Ni) and (Ni+Cu), and uptake of both Ni and Co in (Ni+Co).
Cr uptake was significantly affected by all its coexisting metal ions (Mn, Ni, Co, Cu
and Zn); Mn uptake by Cr, Ni, Co and Cu; Ni by Cr, Co and Cu; Co by Cu and Zn
AND Zn uptake by Mn, Ni and Co in their respective binary combinations.

Multiple regression analyses (Tables 4.81-4.82) showed correlations between
heavy metal uptake in roots and the metals in different binary combinations.
Antagonistic interactions were observed in (Cr+Ni) and (Cr+Co) where Ni and Co
facilitated the uptake of Cr, but the antagonism between Cr-Ni and Cr-Co, inhibited
the uptake of Cr. Similarly, Ni and Co independently increased the uptake of Mn but the
antagonistic interaction between Mn-Ni and Mn-Co reduced the uptake of Mn.
Antagonistic interaction was also observed in (Ni+Co) and (Ni+Zn), where the uptake
of Ni was inhibited by the antagonism between Ni:Co and Ni:Zn. In a few binary
combinations, mixed interactions were observed where the impact of one metal ion on
the uptake potential of the other was more. In (Cr+Mn), (Cr+Cu) and (Cr+Zn), Mn, Cu
and Zn inhibited the uptake of Cr. In (Mn+Co) and (Mn+Zn), the uptake Co and Zn was
inhibited by Mn, whereas in (Mn+Cu), Mn uptake was inhibited by Cu. In (Co+Cu),
increasing concentration of Cu inhibited the uptake of Co but the impact of Co was
higher that promoted its uptake in the roots. A comparison of $\beta$-regression coefficients
of all the heavy metals showed that maximum inhibitory effect was caused by Cu and
Co in (Cu+Co) combinations.

Leaf

The results of metal uptake analysis of leaves of *B. juncea* plants are presented
in Table 4.83 and Fig. 4.19. Zn and Mn showed maximum uptake in leaves, being 0.152
mg g$^{-1}$ dw and 0.11 mg g$^{-1}$ dw respectively at 100 mg kg$^{-1}$ treatment in soil. The least
accumulated metals were Cr and Co, both showing concentrations of 0.007 mg g$^{-1}$ dw at
100 mg kg$^{-1}$ soil concentrations. The order of uptake of heavy metals in leaves was
observed to be, Zn>Mn>Cu>Ni>Co>Cr. Two-way ANOVA (Table 4.84) reveals that
there are statistically significant differences among the mean content values of Cr in the
leaf with respect to the binary combinations (Cr+Ni) and (Cr+Co) in the soil.
Significant differences among mean content values of Mn in leaf with respect to the

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binary combinations (Cr+MN), (Mn+Cu) and (Mn+Zn) were also observed. There were also statistically significant differences among the mean content values of Co in leaf tissues with respect to all the binary combinations with other heavy metals. Differences among mean content values of Cu and Zn in leaves with respect to their binary combination were also observed. Mn uptake in the leaves was significantly affected by Ni and Co and the interaction between (Cr+Zn) and (Mn+Co) significantly affected the uptake of Zn and Co respectively.

Multiple regression analyses (Tables 4.85-4.86) of metal uptake in leaves as a function of heavy metals in binary combinations revealed that the metal accumulation in stem was favored by the antagonistic interactions between the coexisting metal ions of the binary combinations showing significant correlations. In (Cr+Mn), although both the metal ions independently facilitated the uptake of each other, the antagonism between the two inhibited their uptake in the stem. In (Cr+Co), uptake of Cr was increased by Co but the antagonistic interaction between Cr-Co prevented the uptake of Cr. Similar trend was also observed in the uptake of Zn in (Cr+Zn), Ni in (Mn+Ni) and uptake of Co in (Mn+Co), where the antagonism between Cr-Zn, Mn-Ni and Mn-Co inhibited the uptake of Zn, Ni and Co respectively. Among all the heavy metals, maximum inhibitory effect was caused by Mn to the uptake of Co in (Mn+Co) combination.

**Stem**

In the stems of *B. juncea* plants, maximum uptake was shown by Zn (0.188 mg g\(^{-1}\)dw) followed by Mn (0.124 mg g\(^{-1}\)dw). Co and Cr were observed to be least accumulated only 0.007 mg g\(^{-1}\)dw and 0.009 mg g\(^{-1}\)dw, even at the highest concentration of 100 mg kg\(^{-1}\) (Table 4.87, Fig. 4.20). The order of accumulation of heavy metals was observed to be, Zn>Mn>Cu>Ni>Cr>Co. Two-way ANOVA (Table 4.88) reveals that there are statistically significant differences among mean content values of all the metals in the stem with respect to their respective binary combinations in the soil, except for Cr uptake in (Cr+Mn), Mn uptake in (Mn+Co), (Mn+Cu); Ni uptake in (Ni+Cu) and Cu uptake in (Cu+Zn). Mn uptake was significantly affected by Cr in (Cr+Mn), by Ni in (Mn+Ni) and by Zn in (Mn+Zn). Cu and Zn uptake was significantly affected by Ni in (Ni+Cu) and by Cu in (Cu+Zn) respectively. The
interaction between (Ni+Cr) and (Ni+Co) significantly affected the uptake of Cr and Co respectively.

Multiple regression analyses (Tables 4.89-4.90) showed few significant correlations between metal uptake in stem and the metals in binary combinations and further revealed the interactions occurring between the metal ions. In (Cr+Mn) and (Mn+Zn), the uptake of Mn was facilitated by Cr and Zn independently, but the antagonistic interactions between both the ions, inhibited the uptake of Mn. A strong correlation was observed in the uptake of Cr in (Cr+Co), where a mixed interaction occurred between Cr and Co. Increasing concentrations of Co prevented the uptake of Cr. In (Mn+Co) combination, synergism occurred between Mn and Co, where increasing concentration of Mn promoted the uptake of Co. The maximum inhibitory effect was caused by Co to the uptake of Cr in (Cr+Co), whereas maximum stimulatory effect was caused by Mn to the uptake of Co in (Mn+Co).

Fruit

Table 4.91 and Fig. 4.21 present the results obtained from the metal uptake analysis of fruits of *B. juncea* plants grown in heavy metal treatments. Zn and Mn showed maximum uptake, being 0.018 mg g⁻¹ dw and 0.017 mg g⁻¹ dw respectively at 100 mg kg⁻¹ whereas, Cr showed the least accumulation (0.002 mg g⁻¹ dw). The uptake of metals in the fruits of *B. juncea* plants was observed to be very less as compared to the other parts of the plants. Two-way ANOVA (Table 4.92) reveals that there are statistically significant differences among value concentrations of almost all the metals in the fruits with respect to the respective binary treatments in the soil. Co and Ni uptake was significantly affected by Cr and Zn, Cu uptake by Mn and Zn, and Zn uptake was significantly affected by all the metal ions in their respective combinations (except for Mn). In most of the binary combinations, interactions between the coexisting metal ions also significantly affected the uptake of each other.

Multiple regression analysis (Table 4.93) and multiple regression interaction model (Table 4.94) revealed few significant correlations between the heavy metal uptake in fruits and the corresponding binary combinations. In (Cr+Ni), uptake of Cr was inhibited by increasing concentrations of both the metals, however, the antagonism
between Cr and Ni facilitated the uptake of Cr. In (Ni +Cu), although the increasing concentration of both the metals facilitated the uptake of Ni, the antagonistic interaction between them reduced the uptake of Ni.

**Total metal uptake**

Total metal uptake in the plants of *B. juncea* grown in binary combinations of heavy metals is presented in Table 4.95. Zn showed maximum uptake (1.951 g plant⁻¹) followed by Mn (1.444 g plant⁻¹) at 100 mg kg⁻¹ treatment, whereas Cr showed the least accumulation, being only 0.147 g plant⁻¹.

Multiple regression analyses (Tables 4.96-4.97) revealed few significant correlations between metal uptake and the corresponding binary treatments, and the metal interactions occurring between the respective ions. In (Cr+Co) although Co facilitated the uptake of Cr, the antagonism between Cr and Co inhibited the uptake of Cr in the plants. Similar antagonistic interaction was also observed in (Mn+Ni) combination where Ni uptake was decreased due to the antagonism between Mn and Ni. The uptake of Zn in (Cr+Zn), (Ni+Zn) and (Co+Zn) was inhibited as Cr, Ni and Co decreased the Zn uptake due to a mixed interaction, where Zn act synergistically to its coexisting ions leading to its decreased uptake. Also in (Mn+Ni), Mn uptake was reduced because of the inhibitory effect of Ni. The uptake of Ni was facilitated by Mn but the antagonistic interaction occurring between them caused decrease in Ni uptake.
4.3.2 Ternary combinations

4.3.2.1 Shoot emergence

The effect of heavy metals in ternary combinations is presented in Table 4.98, Fig. 4.22. It was observed that the presence of higher concentrations of heavy metal significantly decreased the seedling emergence. Maximum inhibition to seedling emergence was caused by Cr (5.6%) at 200 mg kg\(^{-1}\). However, presence of Zn significantly increased the seedling emergence to 77.8%. The order of inhibition by different heavy metals applied in higher concentration (200 mg kg\(^{-1}\)) to seedling emergence was observed to be, Cr>Ni>Co>Cu>Mn>Zn.

Multiple regression analysis (Table 4.99) revealed strong correlation between seedling emergence and all the ternary metal combinations. Zn exerted positive effect on seedling emergence in all the ternary combinations, except in (Zn+Mn+Co). Similarly, Mn also showed stimulatory effect in all it ternary combinations with other heavy metals. However, Mn in the presence of Zn produced deleterious effect. In all the ternary combinations maximum inhibitory effect was caused by Ni and Cr.

4.3.2.2 Shoot length

Maximum stimulatory effect on the shoot length was caused by Zn and Mn (Table 4.100, Fig. 4.23), whereas Cr at 200 mg kg\(^{-1}\) caused maximum retardation (1.77 cm) of shoot length of *B. juncea* plants. The order of inhibition to shoot growth by different heavy metals was observed to be, Cr>Cu>Co>Ni>Mn∼Zn.

Multiple regression analysis (Table 4.101) showed strong correlations between shoot lengths and all the ternary combinations of heavy metals. Zn and Mn were observed to cause stimulatory effect in all the ternary combinations, except for (Zn+Mn+Ni), (Zn+Mn+Co) and (Mn+Ni+Co). It was observed that in the presence of Mn:Ni and Mn: Co together, Zn was ineffective in overcoming the toxicity of Ni and Co. Also, Mn was found to be ineffective in the presence of Zn:Ni, Ni:Co and Zn:Co together. Ni showed its maximum toxicity in (Zn+Mn+Ni) and Cr showed its maximum toxicity in (Zn+Mn+Co). Among all the ternary combinations, Cu was found to be most toxic in (Zn+Mn+Cu). Cr showed maximum deleterious effects in (Mn+Co+Cr).
4.3.2.3 Biomass

The biomass of different parts as well as total biomass of *B. juncea* plants is given in Table 4.102 and Figs. 4.24-4.28.

**Root biomass**

Among all the heavy metals, Zn and Mn caused least inhibitory effect on root biomass even at the highest concentration of 200 mg kg$^{-1}$. Cr reduced the root biomass to 0.89 g plant$^{-1}$ at 200 mg kg$^{-1}$ of Cr application. The order of inhibitory effect of heavy metals on root biomass was found to be, Cr>Cu>Ni>Co>Mn>Zn.

Zn and Mn exerted positive effects on root biomass in all their ternary combinations (Table 4.103). Maximum stimulatory effect of Zn and Mn was observed in (Zn+Cu+Cr) and (Mn+Cu+Cr) respectively. However, when Zn and Mn were present together in ternary combination, they showed less effectiveness in alleviating the toxicity of other heavy metals. Ni showed maximum toxic effect in (Zn+Mn+Ni), whereas it showed stimulatory effect in (Zn+Ni+Cu) and (Mn+Ni+Co). Co was found to exert positive effect in (Mn+Co+Cr), whereas in showed maximum toxic effect in (Zn+Mn+Co). Cu affected the biomass in most of its ternary combinations, except for (Zn+Mn+Cu) and (Zn+Ni+Cu). Cr produced higher toxic effects as compared to other heavy metals, the maximum being in (Mn+Ni+Cr) combination.

**Leaf biomass**

At the highest concentration of 200 mg kg$^{-1}$, Mn showed the maximum stimulatory effect on leaf biomass (*i.e.* 3.21 g plant$^{-1}$), as compared to the control. At both the concentrations, 100 mg kg$^{-1}$ and 200 mg kg$^{-1}$, Cr emerged as the most toxic. The order of toxicity of heavy metals, with respect to their effect on leaf biomass was as follows, Cr>Co>Ni>Zn>Mn.

In all the ternary combinations, Zn and Mn exerted positive effect on leaf biomass (Table 4.103), particularly in ternary combinations (Zn+Ni+Co) and (Mn+Ni+Cr) respectively. Ni was found to exert negative effect on leaf biomass in all its ternary combinations except in (Mn+Ni+Co). Maximum inhibitory effect of Ni was observed in (Zn+Mn+Ni). Co showed maximum toxic effect in the presence of both Zn and Mn in (Zn+Mn+Co) combination, whereas in (Mn+Co+Cu), Co exerted positive
effect. However, in (Mn+Co+Cu), its toxicity is greatly reduced. Cu produced maximum toxic effect in (Zn+Mn+Cu).

**Stem Biomass**

Maximum stem biomass of *B. juncea* plants was observed in the treatment of Mn 100 mg kg\(^{-1}\), being 4.62 g plant\(^{-1}\) followed by 4.27 g plant\(^{-1}\) at Zn 100 mg kg\(^{-1}\), whereas in the presence of Cr 200 mg kg\(^{-1}\) the stem biomass was reduced to 1.1 g plant\(^{-1}\). The reduction in stem biomass by different heavy metals followed the order, Cr>Co>Ni>Cu>Zn>Mn.

Multiple regression analysis (Table 4.103) showed positive effect of Zn and Mn in all the ternary treatments. Zn showed maximum stimulatory effect on stem biomass in the ternary combination (Zn+Ni+Cr), whereas Mn was found to be most effective in (Zn+Mn+Ni) and (Mn+Ni+Cr). Ni showed maximum inhibitory effect in (Zn+Mn+Ni), whereas in the presence of Cr, in the ternary combinations (Mn+Ni+Cr) and (Zn+Ni+Cr), Ni exerted less toxic effect. Similarly, Co was found to be more toxic in the ternary combination (Zn+Co+Mn), whereas the presence of Cr, in (Zn+Co+Cr) and (Mn+Co+Cr), lowered its toxic effect. In (Zn+Mn+Cu), Cu produced maximum deleterious effect as compared to its other ternary combinations. However, Cr tended to suppress its toxicity in (Zn+Cu+Cr) and (Mn+Cu+Cr). Cr exerted maximum negative effects among all the heavy metals, particularly in (Zn+Mn+Cr). Further it was observed that Zn and Mn, when present together in a ternary combination were less effective in alleviating the toxicity of Ni, Co, Cu and Cr. Moreover, the toxicity of Ni, Co and Cu was suppressed by Cr which is having greater toxicity than these metals.

**Fruit biomass**

At 100 mg kg\(^{-1}\) of Zn and Mn, fruits biomass was significantly increased by 5.8% and 19.0% respectively as compared to the control. However, at 200 mg kg\(^{-1}\) Cr, fruit biomass was reduced to 0.65 g plant\(^{-1}\). Multiple regression analysis (Table 4.103) revealed that Zn and Mn, in all ternary combinations exerted positive effect, except for (Zn+Mn+Ni) and (Zn+Mn+Co) combinations. Cr caused greater inhibitory effects on the fruit biomass as compared to other heavy metals, maximum being in (Zn+Mn+Cr) combination.
**Total plant biomass**

Among all the heavy metals, Zn and Mn acted as antidotes to other heavy metals and showed positive effect on the plant biomass, whereas increasing concentration of all the other heavy metals caused retardation in the plant growth. Maximum increase in plant biomass (11.16 g plant⁻¹) was observed in the presence of Mn (100 mg kg⁻¹). Cr was found to be the most toxic element that reduced the plant biomass to 3.62 g plant⁻¹.

Significant correlations were obtained between the total plant biomass and the different heavy metals in ternary combinations (Table 4.103). Among all the heavy metals Zn caused stimulatory effect on the total plant biomass as indicated by its positive values of β-regression coefficients.

### 4.3.2.4 Ternary Metal Uptake

The result of metal uptake analysis of different parts of *B. juncea* plants is presented in Table 4.104.

**Roots uptake**

In single metal application, maximum uptake was shown by the Zn (0.144 mg g⁻¹ dw) at 100 mg kg⁻¹ followed by Mn (0.135 mg g⁻¹ dw). The metal least accumulated in the roots was Cr, being 0.62 mg g⁻¹ dw at 100 mg kg⁻¹ in soil. The extent of accumulation of different metals in the roots followed the order, Zn>Mn>Ni>Cu>Co>Cr. Regarding uptake in ternary combinations, Zn and Mn showed maximum accumulation (0.142 and 0.147 mg g⁻¹ dw) in the roots of plants grown in ternary combinations (Zn200+Mn100+Co100) and (Mn100+Ni200+Co100) respectively. Maximum uptake of Ni (0.131 mg g⁻¹ dw) was observed in (Zn100+Ni200+Cu100), whereas Co showed its highest uptake (0.105 mg g⁻¹ dw) in the roots of plants grown in (Mn100+Co200+Cr100). Maximum accumulation of Cu (0.104 mg g⁻¹ dw) and Cr (0.054 mg g⁻¹ dw) was observed in the ternary combinations (Mn100+Cu100+Cr100) and (Zn100+Co100+Cr100) respectively.

Multiple regression analysis (Table 4.105) of heavy metal uptake as a function of different ternary combinations showed several significant correlations. In (Zn+Mn+Ni) uptake of Zn is inhibited by the increasing concentrations of Ni and Zn itself. However, Mn promoted its uptake. The uptake of Ni was also inhibited by its
own increasing concentrations and Mn, whereas Zn facilitated its uptake. In (Zn+Mn+Co) increasing concentrations of Mn and Co decreased the uptake of Co, whereas Zn exerted positive effect on its uptake. In (Zn+Mn+Cu), uptake of Zn was strongly inhibited by Mn and Cu both, whereas Mn uptake was strongly inhibited by Cu. The uptake of each element in (Zn+Mn+Cr) showed strong correlation with the other metal in the combination, as Cr strongly inhibited the uptake of both Zn and Mn, whereas inhibition to Cr uptake was maximum by Zn. In (Zn+Ni+Co), Zn uptake was inhibited by both Ni and Co, whereas Ni uptake was strongly decreased by Co. In (Zn+Ni+Cu), Ni and Cu decreased the uptake of Zn, Co decreased the uptake of Ni, and Co uptake was inhibited both by Zn and Ni. In (Zn+Ni+Cr), increasing concentrations of all the three elements decreased the uptake of Zn. The uptake of Ni was facilitated by Zn but strongly inhibited by Cr, whereas Cr uptake was inhibited by both Zn and Ni. Cu inhibited the uptake of Zn and Co in (Zn+Co+Cu), in turn its own uptake was reduced, both by Zn and Co, Co causing maximum inhibition. However, Zn and Co facilitated the uptake of each other, Cr causing maximum inhibition to the uptake of Zn and Co in (Zn+Co+Cr). Zn also slightly decreased the Co uptake. However, Cr uptake was strongly inhibited by Zn, but the influence of Co was positive on Cr uptake. In (Zn+Cu+Cr), Cr strongly inhibited the uptake of both Zn and Cu, whereas both Zn and Cu decreased the uptake of Cr. Cu decreased the uptake of Zn whereas Zn facilitated its uptake. In (Mn+Ni+Co), Ni facilitated the uptake of Mn, whereas Co strongly inhibited its uptake. In (Mn+Ni+Cu), Ni uptake was inhibited by Cu, whereas the uptake of Cu was inhibited by Mn. Mn facilitated the uptake of Ni but inhibited the uptake of Cr in (Mn+Ni+Cr). However the uptake of Mn was decreased both by Ni and Cr, and Cr caused strong inhibition to the uptake of Ni. The uptake of Co was favoured by Mn but strongly inhibited by Cu in (Mn+Co+Cu). In (Mn+Co+Cr), Cr inhibited the uptake of both Mn and Co in turn the uptake of Cr was inhibited by both Mn and Co. However, Mn and Co facilitated the uptake of each other. In (Mn+Cu+Cr) uptake of Mn was inhibited by Cr, whereas Cu facilitated its uptake. Moreover increasing concentration of Mn itself exerted inhibitory effect on its uptake.
Stem uptake

Among all the heavy metals, Zn showed maximum uptake (0.057 mg g⁻¹ dw) followed by Mn (0.042 mg g⁻¹ dw) at 200 mg g⁻¹ in soil, whereas Cr was least accumulated by stem, being 0.015 mg g⁻¹ dw. In ternary combinations, Zn showed maximum uptake (0.058 mg g⁻¹ dw) in the ternary combination (Zn200+Mn100+Ni100) whereas Mn uptake was maximum at (Mn200+Ni100+Co100). Ni accumulation was highest at (Mn200+Ni200+Co100). Co showed maximum accumulation (0.041 mg g⁻¹ dw) in (Mn100+Ni100+Cu200), whereas Cr among all its ternary combinations showed maximum accumulation (0.031 mg g⁻¹ dw) in (Zn200+Co100+Cu200).

The results of multiple regression analysis (Table 4.105) revealed significant correlations between heavy metal uptake and their respective ternary combinations. In (Zn+Mn+Ni), both Zn and Mn mutually facilitated the uptake of each other, whereas Ni decreased the uptake of both Zn and Mn. In (Zn+Mn+Co), the uptake of each element was also strongly affected by the presence of other coexisting elements, as Zn and Mn mutually inhibited the uptake of each other and Co decreased the uptake of both Zn and Mn. Co uptake was strongly inhibited by Zn, whereas Mn facilitated its uptake. In (Zn+Ni+Co) uptake of Ni was strongly inhibited by Co but facilitated by Zn, whereas Ni inhibited the uptake of Zn. Co uptake was decreased by both Zn and Ni. In (Zn+Ni+Cu), Zn uptake was inhibited by Ni but facilitated by Cu. In (Zn+Ni+Cr), Ni and Cr, both decreased the uptake of Zn but Zn facilitated the uptake of Ni. In (Zn+Co+Cu), Cu strongly inhibited the uptake of Zn, whereas Zn facilitated but Co inhibited its uptake. In (Zn+Co+Cr), Co and Cr, both retarded the uptake of Zn. Similarly, Co uptake was inhibited both by Zn and Cr. In (Zn+Cu+Cr), uptake of Cu was inhibited by Zn and by its own increasing concentrations, whereas Cr promoted its uptake, and Cr uptake was reduced by Cu. In (Mn+Ni+Cu), uptake of Mn was inhibited both by Ni and Cu whereas, uptake of Cu was inhibited by Mn, but promoted by Ni. In (Mn+Ni+Cr) and (Mn+Co+Cu), uptake of Mn was inhibited by Ni, Cr, Co and Cu, whereas uptake of Cu in (Mn+Co+Cr) was facilitated by Co, but inhibited by Mn. In (Mn+Co+Cr), all the three ions inhibited the uptake of each other. In (Mn+Cu+Cr), Mn decreased the uptake of both Cu and Cr, whereas Cu decreased the uptake of Mn. Cu and Cr both facilitated the uptake of each other.
Leaf uptake

Zn (0.089 mg g\(^{-1}\) dw), Mn (0.085 mg g\(^{-1}\) dw) and Ni (0.084 mg g\(^{-1}\) dw) showed greater uptake in the leaves of *B. juncea* plants as compared to other heavy metals. Cr showed only 0.024 mg g\(^{-1}\) dw. In combinations, highest uptake of Zn (0.086) and Mn (0.085 mg g\(^{-1}\) dw) was observed at (Zn\(_{200}\)+Mn\(_{100}\)+Ni\(_{100}\)) and (Mn\(_{100}\)+Ni\(_{200}\)+Co\(_{100}\)) respectively. Ni and Co showed maximum uptake in (Mn\(_{100}\)+Ni\(_{200}\)+Cr\(_{100}\)) and (Zn\(_{200}\)+Mn\(_{100}\)+Cu\(_{200}\)) respectively. Highest uptake of Cu (0.055 mg g\(^{-1}\) dw) was observed at (Zn\(_{200}\)+Mn\(_{100}\)+Cu\(_{200}\)) and Cr showed maximum accumulation (0.051 mg g\(^{-1}\) dw) in (Zn\(_{100}\)+Mn\(_{200}\)+Cr\(_{100}\)). Multiple regression analysis (Table 4.105) showed significant correlation between metal uptake and the corresponding ternary metal combinations. Mn uptake was inhibited both by Zn and Ni in the combinations (Zn+Mn+Ni). In (Zn+Mn+Co), the uptake of Mn was inhibited both by Zn and Co. Also Co uptake was inhibited both by Zn and Mn. Similarly, Mn uptake was retarded both by Zn and Cr in (Zn+Mn+Cr). In (Zn+Ni+Co), Zn decreased the uptake of both Ni and Co, and Ni and Co mutually inhibited the uptake of each other. However, all the three metals inhibited the uptake of each other in (Zn+Ni+Cu). In (Zn+Ni+Cr), Ni uptake was also significantly reduced by both its coexisting ions. Co and Cu mutually inhibited the uptake of each other in (Zn+Co+Cu). Though Zn decreased the uptake of both Co and Cu, Zn uptake was not significantly affected by these ions in the combination. In (Zn+Co+Cr), only Co uptake was significantly decreased by Zn and Cr. Zn significantly inhibited the uptake of both Cu and Cr in (Zn+Cu+Cr). Cr also strongly retarded Cu uptake, whereas Cu facilitated the uptake of Cr. In (Mn+Ni+Co) and (Mn+Ni+Cu), the uptake of Mn was strongly inhibited by both Ni and Co, and Ni and Cu. In turn, Mn also significantly inhibited their uptake. Ni and Co, and Ni and Cu also caused mutual inhibition to each other’s uptake. In (Mn+Ni+Cr), Ni strongly facilitated Mn uptake, whereas Mn inhibited its uptake. Cr significantly reduced the uptake of both the ions. Mn uptake was also significantly inhibited by its coexisting ions in the combinations (Mn+Co+Cu), (Mn+Co+Cr) and (Mn+Cu+Cr). Co uptake was facilitated by Cu but inhibited by both Mn and Cr. Cu uptake was strongly inhibited by both Co
and Cr. Though Mn significantly retarded the uptake of Cr, both Co and Cu facilitated its uptake.

Total metal uptake

Mn and Zn showed highest total metal uptake, being 0.680 g plant\(^{-1}\) in the combination (Zn100+Mn200+Ni100) mg kg\(^{-1}\) and 0.810 g plant\(^{-1}\) in the combination (Zn200+Mn200+Ni100) mg kg\(^{-1}\) respectively (Table 4.106). Multiple regression analysis (Table 4.107) showed significant correlation between metal uptake and the corresponding metal in ternary combinations. In (Zn+Mn+Ni) both Zn and Mn favored the uptake of each other. Ni uptake was favored by Zn but inhibited by Mn. Ni inhibited the uptake of both Zn and Mn. In (Zn+Mn+Co) Mn uptake was inhibited by both Zn and Co. Co uptake was favored by Mn but inhibited by Zn. In (Zn+Mn+Cr), both Zn and Mn inhibited the uptake of each other, and Cr uptake was facilitated by Mn but inhibited Zn. In turn, Cr inhibited the uptake of both Zn and Mn. In (Zn+Ni+Co), uptake of Zn was inhibited both by Ni and Co, whereas Ni uptake was facilitated by Zn but strongly inhibited by Co. In (Zn+Ni+Cu), Ni inhibited the uptake of Zn but Zn facilitated the uptake of Ni. Cu inhibited the uptake of both Zn and Ni. Zn facilitated the uptake of Cu but Ni inhibited its uptake. In (Zn+Ni+Cr), Zn uptake was inhibited by Cu whereas Cr uptake was inhibited by Zn but inhibited by Ni. In (Zn+Co+Cu), Co inhibited the uptake of both Zn and Cu. Zn facilitated the uptake of Co. Cu inhibited the uptake of both Zn and Co. Cu uptake was facilitated by Zn but inhibited by Co. In (Zn+Cu+Cr), Zn uptake was inhibited by both Cu and Cr, whereas Cu uptake was favored by Zn but inhibited by Cr. Both Zn and Cu favored the uptake of Cr. In (Mn+Ni+Co), (Mn+Ni+Cu) and (Mn+Co+Cu), Mn uptake was inhibited by its coexisting Ni, Co and Cu ions. In (Mn+Ni+Cr), both Mn and Ni facilitated the uptake of each other, whereas Cr strongly inhibited the uptake of both the ions. In (Mn+Co+Cr), the uptake of Mn was retarded by both Co and Cr. Co uptake was facilitated by Mn but strongly inhibited by Cr, and Cr uptake was facilitated by Co but inhibited by Mn. In (Mn+Cu+Cr), Mn uptake was strongly inhibited by both Cu and Cr.
4.3.3 Six metal combinations

4.3.3.1 Seedling emergence

Seedling emergence of *B. juncea* seeds was found to be drastically reduced in the presence of all the six metals in varying concentrations (Table 4.108). Multiple regression analysis (Table 4.109) revealed that in six metal mixtures, all the heavy metals retarded the seedling emergence as indicated by their negative $\beta$-regression coefficients. Cr caused the maximum deleterious effect, whereas Ni showed positive effect on the seedling emergence in the presence of all the six metals.

4.3.3.2 Biomass of plants

The combined effect of all the six heavy metals on the biomass of different parts of *B. juncea* plants is presented in Table 4.110.

**Root biomass**

Maximum root biomass (1.58 g plant$^{-1}$) was obtained from the plants grown in the combination (Cr100+Cu50+Co100+Ni50+Mn100+Zn200) and the most deleterious effect on root biomass (0.83 g plant$^{-1}$) was caused in the presence of 100 mg kg$^{-1}$ of each heavy metal in the combination (Cr100+Cu100+Co100+Ni100+Mn100+Zn100). Multiple regression analysis (Table 4.111) revealed negative $\beta$-regression coefficients of all the six heavy metals, thereby indicating their negative effect on root biomass, maximum retardation being caused by Ni.

**Stem biomass**

Among all the six metal combinations, the combinations (Cr50+Cu50+Co50+Ni50+Mn100+Zn100), was found to be least toxic, producing stem biomass of 3.27 g plant$^{-1}$, whereas the combination (Cr100+Cu50+Co100+Ni100+Mn200+Zn200) caused maximum reduction, producing only 1.37 g plant$^{-1}$ of stem biomass. A strong correlation was observed between stem biomass and the different six metal combinations (Table 4.111). Cr, Ni and Mn were found to exert negative effect, whereas Cu, Co and Zn showed positive effect on stem biomass.
Leaf biomass

Maximum production of leaf biomass (3.15 g plant$^{-1}$) was obtained in the combination (Cr100+Cu50+Co50+Ni100+Mn200+Zn200). The combination (Cr100+Cu50+Co100+Ni100+Mn200+Zn100) reduced the leaf biomass to only 1.44 g plant$^{-1}$. All the heavy metals except Zn and Mn, were found to cause negative effect on the leaf biomass, maximum being caused by Cr (Table 4.111).

Total Biomass

The combination (Cr50+Cu50+Co50+Ni50+Zn200+Mn100) was found to be least toxic to B. juncea, producing a total biomass of 7.45 g plant$^{-1}$. Multiple regression analysis (Table 4.111) showed negative $\beta$-regression coefficients for all the heavy metals except for Zn and Mn, indicating thereby, deleterious effects of Cr, Ni, Co and Cu, maximum being of that of Cr, whereas Zn and Mn produced stimulatory effect on the total biomass of B. juncea plants.

4.3.3.3 Heavy metal uptake

The uptake of heavy metals in different parts of B. juncea plants grown in six metal combinations of heavy metals are presented in Tables 4.112-4.114.

Roots

Cr showed maximum uptake of (0.095 mg g$^{-1}$ dw) in the combinations (Cr50+Cu50+Co100+Ni50+Mn100+Zn100) and (Cr50+Cu50+Co50+Ni100+Mn100+Zn100), Cu and Ni showed maximum uptake (0.099 mg g$^{-1}$ dw) in (Cr100+Cu50+Co50+Ni50+Mn100+Zn100) and (Cr50+Cu50+Co100+Ni50+Mn100+Zn200) respectively. Maximum uptake of Co was observed in (Cr100+Cu100+Co50+Ni100+Mn100+Zn100) and (Cr50+Cu50+Co100+Ni50+Mn100+Zn200) respectively. Maximum uptake of Mn was observed in the combination (Cr50+Cu100+Co50+Ni50+Mn200+Zn200), whereas Zn showed maximum uptake in the combination (Cr50+Cu100+Co50+Ni50+Mn200+Zn100).

Multiple regression analysis (Table 4.115) showed significant correlation between the uptake of a particular heavy metal in the roots and the six metal combinations. Cu uptake was inhibited by all except Cr and Co. Co uptake was inhibited by Cu and Ni, whereas Cr, Zn and Mn facilitated its uptake. Ni uptake was
facilitated by all the other heavy metal but except that by Cu. Except Cr and Co, Mn uptake was also facilitated by all the other heavy metals. Zn uptake was facilitated by all heavy metals, except for Mn.

**Stem uptake**

Maximum uptake of Cr was observed in the combinations (Cr100+Cu50+Co50+Ni100+Mn100+Zn200) and (Cr100+Cu50+Co100+Ni50+Mn200+Zn200). Cu showed highest uptake in (Cr50+Cu100+Co50+Ni50+Mn100+Zn100). Maximum uptake of Co was observed in 3 combinations viz., (Cr100+Cu100+Co100+Ni100+Mn100+Zn200), (Cr100+Cu100+Co100+Ni50+Mn200+Zn200) and (Cr50+Cu50+Co50+Ni100+Mn200+Zn200). Ni showed highest uptake in (Cr100+Cu100+Co50+Ni100+Mn100+Zn200). Maximum uptake of Mn was observed in the combination (Cr50+Cu100+Co50+Ni50+Mn200+Zn200). Zn showed highest uptake in 3 combinations viz., (Cr50+Cu50+Co50+Ni50+Mn100+Zn200).

Significant correlations were observed (Table 4.115) between the uptake of each heavy metal and the combination of six heavy metals. Cr uptake was facilitated by all other heavy metals except for Zn and Mn, whereas Cu uptake was inhibited by all other metals except for Ni and Mn. Co uptake was facilitated by all except Cr. Similarly, Ni uptake was also facilitated by all other metals except Cu. Mn uptake was inhibited only by Co and Cr. The uptake of Zn was promoted by all the heavy metals except by Cu.

**Leaf Uptake**

Cr uptake in leaf was highest in the combination (Cr100+Cu50+Co100+Ni100+Mn100+Zn200), whereas Cu showed maximum uptake in (Cr100+Cu100+Co100+Ni50+Mn100+Zn100). Co showed maximum uptake in (Cr50+Cu50+Co100+Ni50+Mn100+Zn200). The uptake of Mn was observed to be highest in combinations (Cr50+Cu100+Co50+Ni50+Mn100+Zn200), (Cr100+Cu100+Co100+Ni50+Mn100+Zn200) and (Cr100+Cu100+Co50+Ni100+Mn100+Zn200), whereas uptake of Zn was maximum in (Cr50+Cu100+Co50+Ni50+Mn100+Zn200).

The uptake of each heavy metal in the leaves was significantly correlated with heavy metal concentrations added to the soil for all the treatments (Table 4.115). Cr uptake was retarded by Co and Zn, whereas Ni strongly facilitated its uptake in the
leaves of *B. juncea* plants. Cr and Mn stimulated the uptake of Cu, whereas Ni, Co and Zn exerted negative effect on its uptake. Ni and Co uptake were promoted by all the metals, except by Mn. Mn uptake was facilitated by all the heavy metals, except by Cr. Zn uptake was facilitated by Cr, Cu and Ni, whereas Co and Mn showed inhibitory effect on its uptake.

**Total metal uptake**

The total uptake of each metal in *B. juncea* plants when applied as six metal combinations is presented in Table 4.116. Zn showed maximum total uptake (0.400 g plant⁻¹) in the combination (Cr100+Cu100+Co100+Ni50+Mn200+Zn200), followed by Ni (0.382 g plant⁻¹) in the combination (Cr100+Cu100+Co100+Ni50+Mn200+Zn200).

Multiple regression analysis (Table 4.117) revealed that the total uptake of each heavy metal was strongly correlated with concentrations of heavy metals applied to the soil in six metal combinations. The uptake of Cr was inhibited by Co, Mn and Zn but facilitated by Cu and Ni. Cu uptake was inhibited by all the metals. Co accumulation was facilitated by Cu and Zn but inhibited by Cr, Mn and Ni. The uptake of Ni was facilitated by all the metals studied, except for Cr and Mn. Mn uptake was inhibited by Cr, Co and Ni but facilitated by Cu and Zn. The uptake of Zn was facilitated by Cr, Cu and Ni, whereas Mn and Co decreased its uptake.
4.4 PROTEIN CONTENT

The protein content in *B. juncea* seedlings grown in water culture containing different binary combinations of heavy metals is presented in Table 4.118. The protein content decreased significantly under metal stress. In single metal treatments, maximum decrease (4.87 mg g\(^{-1}\) fw) was observed at Cr 100 mg l\(^{-1}\), whereas among binary combinations, (Mn100+Ni100) caused greatest reduction in the protein content. One-way ANOVA (Table 4.119) showed that the protein content was significantly different from each other in various binary metal combinations of Zn and Mn with other metals. Binary interaction model (Table 4.120) for the protein content as a function of two metals in binary combinations derived using multiple regression analysis revealed significant correlations. Although the partial and \(\beta\) regression coefficients for all the metals in all the binary treatments were negative, the overall interactive effect of binary treatments on the protein content in the seedlings was found to be positive, thereby showing that Ni, Co, Cu and Cr were antagonistic to Mn and Zn.

4.5 ANTIOXIDATIVE ENZYMES

The observations made on activities of different enzymes in the *B. juncea* seedlings grown in binary combination of Zn and Mn is presented in Table 4.121, Fig. 4.29. Enzyme assays of single metal stressed seedlings showed that there was a significant enhancement of antioxidative enzyme activities (except for catalase) with respect to the controls. Among all the heavy metals, Zn and Mn were observed to be the most effective metals in increasing the activities of all the antioxidative enzymes. One-way ANOVA (Table 4.122) showed that the activities of GR, GPX, APX and SOD were significantly different from each other in various binary metal combinations of both Mn and Zn with other heavy metals.

4.5.1 Superoxide dismutase

Maximum SOD activity (18.0 mM UA/mg protein) was observed for Mn 100 mg l\(^{-1}\). The binary combinations of metals also increased SOD activity. The combination (Mn100+Cu100) was most effective in increasing the SOD activity to 20.8 mM UA/mg protein, followed by (Mn100+Cr 00) combination (10mM UA/mg protein).
Binary interaction using multiple regression analysis (Table 4.123) for the relative activities of SOD as a function of different binary combinations revealed significant correlations between the enzyme activity and the metal concentrations in different binary treatments. Although both the metals in binary combinations of Mn and Zn independently increased the enzyme activities as indicated by their positive $\beta$-regression coefficients, the interactive effect on SOD activity was negative due to the antagonism of Zn and Mn with Ni, Co, Cu and Cr.

4.5.2 Guaiacol peroxidase

All the heavy metals whether applied singly or in binary combination significantly increased the activity of GPX. Among single metal treatments, maximum increase (54.1 mM UA/mg protein) was observed in the seedlings treated with Zn 100 mg l$^{-1}$ followed by Mn 100 mg l$^{-1}$ (51.0 mM UA/mg protein).

Among the binary combinations, (Zn+Co) was most effective in increasing the enzyme activities to 57.5 mM/UA/mg protein. Multiple regression analysis (Table 4.123) revealed positive $\beta$ regression coefficients of all the metals in binary combinations with Zn and Mn, thereby showing their independent stimulatory effect on the induction of GPX activity, however the antagonism between both the metal ions produced negative effect on the enzyme activity.

4.5.3 Catalase

The inhibitory effect of Zn and Mn, whether applied singly or in binary combinations with other metals on the activity of catalase was found to be not significant.

4.5.4 Ascorbate peroxidase

A significant increase in the activity of APX was observed in metal stressed seedlings, grown both in single metal treatments and in binary combinations. In single metal applications, maximum APX activity (9.2 mM UA/protein) was observed in Mn 100 mg l$^{-1}$. Among all the heavy metals, Cr was least effective in enhancing the activities APX. However, Cr in binary combinations with Mn (Mn100+Cr100) was found to be most effective in increasing the enzyme activity to 9.5 mM UA/mg protein.

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The results of multiple regression analysis (Table 4.123) showed positive $\beta$-regression coefficients of all the heavy metals, on GPX activity. However, Zn and Mn were found to be antagonistic to the other heavy metals (Ni, Co, Cu and Cr) in their respective binary combinations, thereby causing a negative interactive effect on the activity of GPX.

4.5.5 Glutathione reductase

Mn at 100 mg l$^{-1}$ was found to cause maximum increase in GR activity (9.56 mM UA/mg protein), whereas Cr showed less effect, being only 4.07 mM UA/mg protein. The activity of GR was also significantly increased in the presence of binary combinations, maximum being 10.9 mM UA/mg protein by the combination (Mn 100+Ni 100).

In all the binary combinations of Mn, the $\beta$-regression coefficients due to all the heavy metals were positive indicating that both the metal ions independently increased the GR activity, but their antagonistic interaction exerted negative effect on the enzyme activity. Zn in all its binary combinations independently decreased the GR activity, whereas other metals (Cr, Ni, Co and Cu) increased the activity. The interaction between Mn and other ions was a mixed interaction, where Mn acted synergistically with Ni, Co, Cu and Cr to generate a positive effect on the enzymatic activity (Table 4.123).