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Research Articles

# EFFECT OF HEAVY METALS ON THE DISTRIBUTION OF P, K, Ca, Mg AND MICRONUTRIENTS IN THE CELLULAR CONSTITUENTS OF COCONUT LEAF

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ABSTRACT

The distribution patterns of P, K, Ca, Mg and micronutrients with special reference to heavy metal treatments have been evaluated in coconut and the results indicated that the P distribution in organic acids, polar compounds, proteins and nucleic acids and polysaccharides has been, influenced by the heavy metals. However, the K content of lipids and pigments, organic acids, cellulose and insoluble residues has been significantly altered due to heavy metal treatment. The Ca concentration in lipids and pigments and organic acids has been significantly depressed by heavy metals. A similar pattern of depression of Mg content has been recorded in nucleic acid, polysaccharides and organic acid fractions.

Among micronutrients, iron content in proteins, cellulose and insoluble fractions increased by heavy metals in general and due to Pb and Cd significantly in pigments. All the seven heavy metals increased the Mn content in polar compounds over control. Cadmium, Bismuth, Chromium and Barium exerted profound increase in Zinc distribution in polysaccharides and insoluble residues over control. There was no change in Zinc concentration with lipids, pigments and organic acids. In respect of Copper there was noticeable change due to heavy metals in pigments, cellulose, polysaccharides and insoluble residues. Copper content increased insoluble residues and pigments.

## INTRODUCTION

The effect of heavy metals on the nutrition of coconut has been extensively studied. Biddappa et al, (1987) have shown the inhibitory role of heavy metals on the uptake and translocation of major nutrients in coconut. The alteration in physiological processes in plants following an excess of heavy metals has been well documented (Foy, Chancy and White, 1978). The association of nutrient ions with various subcellular components is a well established fact because of their prominent role in various biological synthesis. The gradual loss of nutrient elements from stored subcellular components might be due to dissociation, degradation or disappearance (Rathore, Bajaj and Wittwer, 1972). Whatley, Ordin and

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Amon (1951) emphasised the importance of subcellular localisation of nutrients in relation to their functional role in cellular activities. Thus a knowledge of changes in subcellular distribution pattern of nutrients accompanying changes in growth and development due to heavy metals seemed to be a valuable method to define their possible biochemical role. The need for such studies with mammalian tissues indicated that several metallic ions showed characteristic distribution pattern in subcellular organelles (Edwards et al, 1961). The present study was undertaken with a view to evaluate the specific inhibitory/ synergistic effect of heavy metals on the incorporation of nutrient elements in various functional cellular constituents of coconut which further governs the productivity of this crop.

## MATERIALS AND METHODS

A pot culture experiment in which high yielding one year old coconut variety West Coast Tall was grown on pure quartz sand during September 1982. During October 1983, the treatment of heavy metals *viz.*, Pb, Cd, Bi, Cr, Ca, Ti, and Ni were imposed. A control treatment was also kept for comparison. Major, secondary and micronutrients were supplied in the form of normal nutrient solution and replaced once every month throughout the experimental period. The treatments of heavy metals in triplicate were imposed in a completely randomised design (Table I).

The treatments were also replaced once per month by flushing out the old solution with water and replacing it with new solution. The tissue samples from the third youngest leaf were taken from the third highest concentration of heavy metal treatments for the fractionation of the cellular constituents and nutrient distribution in 4 year old coconut plants during May 1986. The third concentration was used due to handling problems with an assumption that the basic process would be almost same in the lower concentrations also with lesser magnitude. Cellular fractionation was carried out by using the procedure described by Brooks, Shaw and Asensi Marfil (1981). The P, K, Ca, Mg, Fe, Mn, Zn and Cu contents in each fraction was estimated in a diacid digest with an atomic absorption spectrophotometer (A 975) as per the procedure described by Jackson (1967). The data were statistically processed in order to evaluate the specific inhibitory role of heavy metals on the nutrient incorporation in various cellular constituents.

Table I. *Treatments of heavy metals and their concentrations*

| Treatment no. | Salts used | Metals | Final conc. ppm of respective metal | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| 1. | $Pb(No_3)_2$ | Pb | 5 | 10 | 20 |
| 2. | $Cd\ Cl_2$ | Cd | 1 | 2 | 4 |
| 3. | $Bi(No_3)_3$ | Bi | 2 | 4 | 8 |
| 4. | $K_2\ Cr_2\ O_7$ | Cr | 5 | 10 | 20 |
| 5. | $Ba\ Cl_2$ | Ba | 5 | 10 | 20 |
| 6. | $Ti\ Cl_3$ | Ti | 5 | 10 | 20 |
| 7. | $Ni(No_3)_2$ | Ni | 2 | 4 | 8 |
| 8. | Control | | 0 | 0 | 0 |

## RESULTS AND DISCUSSION

### Phosphorus

The data on the P concentration in cellular fractions as influenced by the

different heavy metals are summarised in Table II. The results indicate that the P content in lipids and pigment fractions was significantly high in Cd treated plant compared to that of control. Other heavy metals did not influence P content of this fraction significantly. In the case of organic acids Pb, Cd, Cr, Ba and Ni significantly reduced P content. The P content of proteins and pectates, nucleic acid, polysaccharides and acid soluble polar compounds was reduced with all metals with the exception of Ni, in nucleic acid. On the other hand, the P content in cellulose was reduced by Pb, Bi, Cr and Ni treatments. The application of Bi and Ni enhanced the P concentration in insoluble residues while Ti and Cd decreased it significantly.

**Potassium**

The data summarised in Table II gives the K distribution pattern in the cellular fractions as influenced by metal treatments. It is evident that Ni, Ba and Cr enhanced the K content in lipids and pigments while Pb and Cd decreased it significantly. On the other hand, the K content in acid soluble polar compounds, cellulose and pectates and insoluble residues were increased markedly by the metal treatments especially in pectates and insoluble residues. The K content of nucleic acid and polysaccharide fractions has not been affected except Bi in nucleic acid, and Pb and Cd in polysaccharide fractions.

**Calcium**

The data show that the Ca distribution (Table III) in nucleic acid and polysaccharide has not been affected by metal treatments. However, the Ca content of lipids, pigments, and organic acids was depressed by almost all the metals except Pb in lipids and Pb and Bi in organic acids. Cr had a profound influence on the Ca content of acid soluble polar compounds when compared with control and other metals. In the case of proteins and pectates Pb, Cd, Cr and Ni significantly increased the Ca concentration and Cd, Ba and Ti also enhanced Ca in cellulose. The Ca content of insoluble residue was also not significantly altered in all metal treatments except Cd and Bi.

**Magnesium**

There was a decrease of Mg content in most of the cellular fractions as a result of metal application (Table III). The Mg content of lipids, acid soluble polar compounds and proteins and pectates was least affected except by Cd and Cr in polar compounds, Ti and Ni in proteins and Cr and Bi in lipids. The Mg concentration of organic acids, nucleic acids and polysaccharides was significantly decreased by most of the metals except Ba (organic acids) and Ni (polysaccharides) where high concentrations of Mg were recorded. The Mg content in cellulose was increased by most of the metals. On the other hand, Cd, Bi and Ba depressed the Mg content of the insoluble residues significantly.

**Iron**

The data (Table IV) indicates that the iron concentration in pigments is significantly more with Pb and Cd treatments. The Fe distribution in organic acids, polar compounds and polysaccharides has not been altered by heavy metal treatments except Pb in

Table II. *Effect of heavy metals on P and K content in sub-cellular fractions ($\mu g/5g$)*

| Fractions | Constituents | Pb | | Cd | | Bi | | Cr | | Ba | | Ti | | Ni | | Control | | CD5% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | K | P | K | P | K | P | K | P | K | P | K | P | K | P | K | P | K |
| Ethanol | Lipids & pigments | 203 | 1410 | 831 | 1060 | 155 | 4063 | 253 | 5813 | 194 | 4813 | 234 | 3188 | 237 | 7375 | 248 | 3750 | 188 | 1023 |
| Water | Organic acid | 276 | 1470 | 42 | 1030 | 334 | 6063 | 226 | 8500 | 255 | 7563 | 348 | 4625 | 221 | 4625 | 374 | 2625 | 87 | 1510 |
| HCl | Acid soluble polar compounds | 117 | 1250 | 90 | 1010 | 176 | 4063 | 71 | 4013 | 131 | 3813 | 125 | 1250 | 172 | 9625 | 227 | 1500 | 39 | 1167 |
| Acetone insoluble | Proteins & pectates | 13 | 40 | 38 | 29 | 78 | 50 | 110 | 28 | 119 | 33 | 132 | 55 | 125 | 48 | 178 | 27 | 16 | 17 |
| $HClO_4$ | Cellulose & lignins | 143 | 340 | 173 | 700 | 127 | 438 | 125 | 350 | 183 | 533 | 212 | 410 | 159 | 250 | 197 | 240 | 38 | 85 |
| $HClO_4$ insoluble | Nucleic acid | 11 | 9 | 3 | 17 | 73 | 23 | 67 | 15 | 65 | 10 | 69 | 5 | 116 | 18 | 108 | 13 | 17 | 5 |
| NaOH | Proteins & polysaccharides | 166 | 84 | 86 | 110 | 199 | 250 | 240 | 313 | 247 | 298 | 313 | 165 | 432 | 230 | 607 | 240 | 57 | 105 |
| Residue | Insolubles | 84 | 91 | 64 | 84 | 261 | 143 | 97 | 163 | 130 | 125 | 47 | 85 | 176 | 77 | 122 | 40 | 50 | 26 |

Table III. *Effect of heavy metals on Ca and Mg content in sub-cellular fractions (μg/5g)*

| Fractions | Constituents | Pb | | Cd | | Bi | | Cr | | Ba | | Ti | | Ni | | Control | | CD 5% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg | Ca | Mg |
| Ethanol | Lipids & pigments | 1535 | 1347 | 988 | 1312 | 418 | 574 | 690 | 697 | 1095 | 1142 | 664 | 1549 | 1064 | 1355 | 1988 | 1130 | 296 | 569 |
| Water | Organic acid | 2102 | 814 | 972 | 862 | 1795 | 608 | 1075 | 944 | 1505 | 1869 | 1231 | 1172 | 1388 | 1030 | 2010 | 1490 | 374 | 268 |
| HCl | Acid soluble polar compounds | 2333 | 776 | 2867 | 635 | 1996 | 976 | 3274 | 1222 | 2095 | 860 | 2348 | 860 | 1825 | 675 | 2383 | 935 | 778 | 277 |
| Acetone insoluble | Proteins & pectates | 685 | 176 | 854 | 152 | 491 | 148 | 733 | 154 | 450 | 135 | 530 | 62 | 646 | 195 | 455 | 110 | 135 | — |
| $HClO_4$ | Cellulose & lignins | 1268 | 245 | 2334 | 152 | 803 | 350 | 1154 | 170 | 1833 | 588 | 2239 | 662 | 1440 | 430 | 979 | 385 | 417 | 91 |
| $HClO_4$ insoluble | Nucleic acid | 492 | 17 | 441 | 15 | 446 | 33 | 410 | 28 | 421 | 24 | 457 | 29 | 444 | 125 | 449 | 150 | — | 29 |
| NaOH | Proteins & polysaccharides | 718 | 66 | 852 | 69 | 665 | 110 | 714 | 68 | 657 | 116 | 624 | 74 | 978 | 240 | 915 | 185 | — | 44 |
| Residue | Insolubles | 672 | 111 | 767 | 80 | 796 | 52 | 600 | 120 | 599 | 56 | 635 | 86 | 703 | 315 | 493 | 145 | 239 | 61 |

Table IV. *Effect of heavy metals on Fe and Mn content in sub-cellular fractions (μg/5g)*

| Fractions | Constituents | Pb | | Cd | | Bi | | Cr | | Ba | | Ti | | Ni | | Control | | CD 5% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn | Fe | Mn |
| Ethanol | Lipids & pigments | 93 | 8.3 | 69 | 7.2 | 18 | 2.5 | 21 | 3.7 | 23 | 5.4 | 22 | 9.5 | 30 | 2.0 | 11 | 1.8 | 19.2 | — |
| Water | Organic acid | 45 | 15.0 | 20 | 8.0 | 36 | 4.3 | 24 | 8.7 | 37 | 23.4 | 35 | 23.3 | 20 | 1.4 | 30 | 3.3 | 8.3 | 6.1 |
| HCl | Acid soluble polar compounds | 131 | 23.4 | 23 | 12.7 | 55 | 14.7 | 42 | 16.6 | 24 | 17.8 | 26 | 19.4 | 18 | 11.1 | 41 | 13.1 | 24.7 | 7.3 |
| Acetone insoluble | Proteins & pectates | 30 | 1.3 | 39 | 2.7 | 39 | 2.5 | 29 | 1.0 | 31 | 1.2 | 43 | 1.7 | 15 | 0.16 | 9 | 0.12 | 9.7 | — |
| $HClO_4$ | Cellulose & lignins | 81 | 3.8 | 93 | 3.9 | 76 | 5.1 | 51 | 6.0 | 74 | 1.5 | 31 | 4.5 | 44 | 0.73 | 17 | 0.94 | 18 | 1.07 |
| $HClO_4$ insoluble | Nucleic acid | 17 | 0.36 | 12 | 0.54 | 30 | 0.49 | 18 | 0.81 | 24 | 0.84 | 15 | 0.28 | 17 | 0.07 | 28 | 0.09 | 4 | 0.36 |
| NaOH | Proteins & polysaccharides | 87 | 2.90 | 83 | 0.03 | 90 | 0.81 | 101 | 0.78 | 88 | 3.80 | 84 | 5.70 | 118 | 1.40 | 112 | 1.30 | — | 1.87 |
| Residue | Insolubles | 117 | 6.70 | 108 | 5.40 | 118 | 6.20 | 98 | 4.60 | 92 | 2.80 | 72 | 3.70 | 87 | 0.50 | 65 | 0.24 | 22.9 | 2.38 |

Table V. *Effect of heavy metals on Cu and Zn content in sub-cellular fractions (μg/5g)*

| Fractions | Constituents | Pb | | Cd | | Bi | | Cr | | Ba | | Ti | | Ni | | Control | | CD 5% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn | Cu | Zn |
| Ethanol | Lipids & pigments | 8.5 | 18.2 | 3.9 | 3.0 | 5.4 | 2.9 | 5.2 | 4.9 | 6.8 | 6.9 | 4.8 | 7.9 | 3.1 | 7.0 | 1.2 | 5.6 | 2.54 | 3.34 |
| Water | Organic acid | 4.6 | 11.0 | 2.9 | 8.6 | 3.6 | 4.1 | 4.8 | 4.5 | 10.8 | 12.8 | 5.0 | 5.1 | 3.7 | 7.1 | 2.0 | 8.2 | 2.63 | 4.08 |
| HCl | Acid soluble polar compounds | 14.4 | 17.8 | 16.8 | 10.4 | 7.7 | 47.0 | 7.9 | 46.4 | 8.6 | 27.8 | 2.87 | 26.6 | 5.8 | 8.2 | 10.2 | 23.2 | 5.86 | 14.77 |
| Acetone insoluble | Proteins & pectates | 3.4 | 7.7 | 4.7 | 11.8 | 2.3 | 7.7 | 1.8 | 5.7 | 1.8 | 6.0 | 4.0 | 11.5 | 1.7 | 14.7 | 0.84 | 5.2 | 0.98 | 5.03 |
| $HClO_4$ | Cellulose and lignins | 4.1 | 4.3 | 6.0 | 11.5 | 4.2 | 22.1 | 5.4 | 18.6 | 5.9 | 22.0 | 2.2 | 13.5 | 2.7 | 9.0 | 1.4 | 6.1 | 2.21 | 8.56 |
| $HClO_4$ insoluble | Nucleic acid | 0.87 | 1.3 | 0.64 | 3.6 | 4.0 | 3.9 | 1.7 | 2.8 | 1.4 | 2.4 | 2.2 | 3.2 | 1.2 | 9.6 | 1.2 | 11.0 | 1.18 | 2.63 |
| NaOH | Proteins and polysaccharides | 5.0 | 2.5 | 7.7 | 25.6 | 19.4 | 26.6 | 16.6 | 29.9 | 12.5 | 28.1 | 16.8 | 28.1 | 9.4 | 15.4 | 6·2 | 13.8 | 4.68 | 10.63 |
| Residue | Insoluble | 13.4 | 12.3 | 7.1 | 27.2 | 12.2 | 42.6 | 24.0 | 24.7 | 13.2 | 24.9 | 14.8 | 20.3 | 7.7 | 10.7 | 2.5 | 7.9 | 3.41 | 16.64 |

organic acid and polar compounds. The Fe content of proteins, cellulose and insoluble fractions generally increased to a varying degree with different heavy metals. On the other hand the Fe concentration in nucleic acid was depressed significantly by the application of Pb, Cd, Cr, Ti and Ni.

**Manganese**

In general, the Mn content of (Table IV) lipids, pigments and proteins was not significantly influenced by heavy metal treatments. Pb, Ba and Ti enhanced the Mn content of organic acids while all heavy metals increased the Mn concentration in polar compounds. A similar pattern was obtained for Mn distribution in protein, cellulose and insoluble residues. In the case of nucleic acid an increase in Mn content was recorded for Cd, Bi, Cr, Ba and Ti enhanced the Mn content of polysaccharides.

**Zinc**

The zinc concentration in lipids, pigments and organic acids was not affected (Table V) by metal treatments except Pb in lipids and Ba in organic acids. The Zn concentration in the polar compounds was enhanced by Bi and Cr and depressed by Ni. The Zn concentration in proteins and cellulose was influenced by different metals to a varying degree, Cd, Bi, Cr and Ba profoundly increased the Zn distribution in polysaccharides and insoluble residues. On the other hand, the Zn concentration in nucleic acid was suppressed by all heavy metals except Ni.

**Copper**

There was a considerable impact of the heavy metals on the Cu distribution in pigments, cellulose, polysaccharide and insoluble residues. Almost all of the heavy metals increased the Cu content in insoluble residue and pigments and Cr, Ba and Ti affected the Cu content of the organic acids. Only Cd increased the Cu contents of polar compounds and Bi in nucleic acid. In the case of proteins, the Cu content was influenced by Pb, Cd, Bi and Ti and in polysaccharides by Bi, Cr, Ba and Ti.

In general, the nutrient distribution in various functional cellular constituents varied with the heavy metal treatments. This might be due to the specific inhibitory/synergestic role of heavy metals on the enzyme systems which involved in such incorporation. (Foy et al, 1978). The other possible reasons for such differential behaviour could be attributed to the competitive nature, dissociation or displacement, of nutrient element from such functional side (Rathore et al, 1972). However, this needs further investigation to establish specific reaction mechanisms involved in the inhibition of incorporation of nutrient elements in the cellular functional sides.

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