Inorganic elements play an important role in the physiology of parasites. These are intimately related with growth, maintenance of acid-base equilibrium and synthesis of organic material. In present study seven inorganic elements viz. Ca, Mg, Fe, Zn, Cu, Pb and Cd was determined quantitatively in immature, mature and gravid proglottids of Moniezia expansa by atomic absorption spectrophotometry. Quantitative analysis revealed that Ca and Mg content were higher than Fe. Cu and Pb were very low.

Introduction:-
Inorganic elements play an important role in the physiology of parasites. These are intimately related with growth, maintenance of acid-base equilibrium and synthesis of organic material.

Some of these inorganic elements are toxic heavy metals like Cd and Pb which are major health risk concern due to their bioaccumulation potential, persistent nature and harmful biological effects (Sharma et al., 2010) and their presence in parasites may indicate their presence in host environment. Thus, its presence in M.expansa might be used as an accumulation indicators or bioindicator system for heavy metal pollution in terrestrial bioptopes.

Ca is an important element for the integrity of intercellular cement substances (Heilbrunn, 1952). Gingell et al. (1970) believed that both Ca and Mg are essential for "intercellular ionic bridging", changes at the cell surface and shape of the cell during cleavage. Cu and Zn are cofactors associated with a number of oxidative enzymes, dehydrogenases, phosphatases and cytochrome oxidases which have been demonstrated in cestodes (Smyth, 1969; Enigk et al., 1976). Experimental studies have further suggested that Zn is required for initiation of DNA and protein synthesis within the cells (Fujioka and Liberaman, 1964). The availability of Zn within the nucleolus leads to increase mRNA synthesis and this in turn increased availability of enzymes for DNA synthesis or transcription of DNA into RNA (Kay et al., 1969).

The importance of inorganic substances to intestinal helminths is also demonstrated by the influence of mineral deficiencies in the host’s diet (Ackert and Gaafar, 1949; Mathur and Pandey, 1969; Nadakal et al., 1975; Enigk et al., 1973). Thus, the amount of mineral components of these worms depend on the nutritional conditions of the host and their dependency on host’s nutrients is also helpful in their control in host.

Lots of work has been done in this field on trematodes and nematodes but not much in cestodes. Notable contribution are those of Goodchild et al. (1962), Gyu’akhmedov et al. (1976), Singh et al. (1978), Nadakal et al. (1982), Gabrashanska, M. and Damyanova, A. (1987), Jankovska, I. et al (2011, 2010a,b).
No work has been done in India, on inorganic elements in *M. expansa*. So, the present study is an attempt to determine some of the inorganic elements in it by atomic absorption spectrophotometry. Also, this is the first attempt in respect to the study of inorganic elements in different proglottids of *Moniezia expansa*.

**Materials and methods:-**
Specimens of *Moniezia expansa* were procured from the intestines of infected sheep slaughtered at local abattoirs and were washed thoroughly with normal saline until they were free from the debris, washed well with distilled water and separated into immature, mature and gravid regions. Tissues were blotted quickly on filter paper to soak the adhering moisture on body and were weighed. A portion of each sample was weighed and were dried at 100°C ± 5°C for 24 hrs in hot air oven and used for determining the dry weight percentage. The weighed fresh tissues were transferred to conical flasks and 10 ml of digestion mixture (HNO₃ and perchloric acid mixture in 6:1 ratio) was added to all conical flasks including 2 blanks.

The digestion of tissues was done on hot plate in the fuming chamber and the acid was evaporated completely until white fumes appeared.

After digestion, the samples were reconstituted by adding 10 ml of distilled water to each flask. The samples were read for metals Ca, Mg, Fe, Zn, Cu, Pb and Cd on a atomic absorption spectrophotometer model Varian 250 plus against the suitable standards for each metal in the linear range of 0.5 to 5 ppm. Standards used were purchased from Sigma, USA.

**Results:-**
Concentration of inorganic elements in immature, mature and gravid proglottids of *Moniezia expansa* in percentage dry weight of tissues is shown in Table 1. The values are mean ± S.D. of five samples in duplicate.

| Elements | Immature proglottids | Mature proglottids | Gravid Proglottids |
|----------|----------------------|--------------------|--------------------|
| 1. Ca    | 0.035±0.0013         | 0.025±0.009        | 0.046±0.004        |
| 2. Mg    | 0.037±0.005          | 0.035±0.004        | 0.043±0.003        |
| 3. Fe    | 0.0084±0.005         | 0.00075±0.00008    | 0.00062±0.00002    |
| 4. Zn    | 0.0021±0.0001        | 0.0022±0.0002      | 0.0027±0.0004      |
| 5. Cu    | 0.0003±0.00007       | 0.0002±0.0003      | 0.0004±0.00005     |
| 6. Pb    | 0.0005±0.00006       | 0.00004±0.00000001 | 0.0002±0.0001      |
| 7. Cd    | –                    | –                  | –                  |

**Discussion:-**
The results of present study reveals that Ca, Mg, Fe, Pb, Cu and Zn were found in immature, mature and gravid proglottids of *Moniezia expansa* but Cd was found to be absent.

The amount of Ca was 0.035%, 0.025% and 0.046% respectively in immature, mature and gravid proglottids of *M. expansa*. The value of Ca is low in comparison to that reported by Goodchild et al. (1962) in *Hymenolepis diminuta*. Singh et al. (1978) in *Thysaniezia gigardi*, Nadakal et al. (1982) in *Raillietina tetragona*. It has been found that the Ca content is highest in immature than mature region and lowest in gravid regions of different cestodes (Goodchild et al., 1962; Singh et al., 1978; Nadakal et al., 1982). But the present study in *M. expansa* Ca content is found to be the highest in gravid region than in immature and mature region.

The amount of Mg is 0.037%, 0.035% and 0.043% respectively in immature, mature and gravid proglottids of *Moniezia expansa*. It is low in comparison to that of Goodchild et al. (1962) in *Hymenolepis diminuta* and Singh et al. (1978) in *Thysaniezia gigardi*. In the present study, Mg content is highest in gravid region followed by immature and mature regions which is similar to that studied by Goodchild et al. (1962) in *Hymenolepis diminuta*. Mg is reported for the first time in *M. expansa*.

The Fe content in immature, mature and gravid proglottids of *Moniezia expansa* is 0.0084%, 0.00075% and 0.00062% respectively. It is similar to that reported by Jankovska.I. et al (2011) in *M. expansa*. Fe content in
different proglottids is reported for the first time. The Fe content of immature region is much higher than in mature and gravid regions.

The amount of Zn in present study is 0.0021%, 0.0022% and 0.0027% respectively in immature, mature and gravid proglottid of Moniezia expansa. It is low in comparison to that studied by Jankovska,(2011) in M. expansa, Nadakal et al. (1982) in Raillietina tetragona. Zn content in present study is slightly higher in gravid than mature and immature proglottid and the study of Zn in different proglottids of Moniezia expansa is done for the first time.

The amount of Cu is 0.0003%, 0.0002% and 0.0004% respectively in immature, mature and gravid proglottid of Moniezia expansa. This is in accordance to Jankovska,I. et al (2011) who found it 0.00052% in M.expansa. These values were lower in comparison to that reported by Nadakal et al. (1982) in Raillietina tetragona. This may be because Cu content of some parasites seems to depend on the food diet (Von Brand, T., 1979; Bremner,1961; Enigk et al., 1973).

The amount of Lead (Pb) found is 0.0005%, 0.00004% and 0.0002% respectively in immature, mature and gravid proglottids of Moniezia expansa which is similar to that of Jankovska et al (2010) who reported 0.0000145% in M. expansa. It is highest in immature then in gravid and lowest in mature proglottid. Lead is one of the most toxic of the trace elements occurring in food and is not at all an essential constituent of any living organism. So, significance of its presence is not understood, may be possibly due to the contaminated host diet and surroundings as earlier reported for different parasites (Kegley et al. 1970).

Thus, it may be possible that these trace elements present in very small amounts in the environment may be taken in the body of parasite and may constitute a significant part of its chemical composition. Thus these cestode may be a bioindicator of such toxic element in host’s environment (Jankovska,I.2010).

Cadmium is found to be absent in Moniezia expansa in the present study but was reported in the same parasite by Gabrashanska and Damyanova (1987) and Jankovska, I.(2010, 2011).

There are variations observed in the contents of various inorganic elements present in the Moniezia expansa. Ca and Mg contents are higher than Zn which is higher than Fe. Cu and Pb are much low. These variations may be probably due to the amount of these elements in the host's intestinal content as intestinal parasites acquire inorganic substances largely from the intestinal contents of their hosts and gut less parasites acquire these inorganic substances through the surface (Von Brand T, 1973). Several helminthes are able to accumulate considerable concentration of elements from the host body (Barus et al. 2003; Lafferty,1997; Sures,2004)

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Bibliography:
1. Ackert, J.E. and Gaafar, S.M. (1949) Phosphorous deficiency a limiting factor in fowl parasitism. J. Parasitol. 35 (6 Sect. 2): p1
2. Barus,V., Tenora,F., Sunbera,R., (2003) Relative concentrations of four heavy metals in the parasites Protospirura muricola (Nematoda) and Inermicaspifer arvicantidis (Cestoda) in their definitive host silvery mole rat (Heliophibius argeneigereus: Rodentia). Helminthologia, 40:227-232.
3. Enigk, K; Feder, H and Dey Hazra, A. (1976) Mineral content and enzyme activity of Cysticercus tenuicollis in the sheep and pig. Zbl. Vet. Med. B23: 255-264.
4. Fujioka and Liberman, I. (1964) A Zn" requirement for synthesis of deoxyribonucleic acid by rat liver. J Biol. Chem. 239: 1164-1167.
5. Gabrashanska, M. and Damyanova, A. (1987) Comparative investigations of the mineral content of helminths (Fasciola hepatica, Moniezia expansa, Ascaridia galli and Paramphistomum sp. Khelminologya, 24: 12-19.
6. Gingell,D.; Garrod, D.R. and Palmer, J.F. (1970) Divalent cations and cell adhesion. In: Calcium and cellular function. (Editor Cuthbert, A.W.) Macmillan, London.
7. Goodchild, C.G.; Dennis, E.S. and Moore, J.D (1962) Flame photometric studies of helminths: calcium, magnesium, potassium and sodium in Hymenopilis diminuta. Exp. Parasitol. 12: 107-113.
8. Greichus, A. and Greichus, Y.A. (1980) Identification and qualification of some elements in the hog roundworm Ascaris lumbricoides suum and certain tissues of its host. Int. J. Parasitol. 10: 89-91.
9. Gyul'akhmedov, A.N., Sadykhov, I.A. and Khalilova, K.H.A. (1976) Selenium content in Moniezia expansa and Fasciola hepatica. In: Selen v biologii. Kniga 2. Baku, USSR; "ELM". 148-149.
10. Heilbrunn, L.V. (1952) An outline of General Physiology. W.B. Savenders Co., Philadelphia.
11. Jankovskva,I., Lukesova,D., Szakova,J., Langrova,I., Vadlejch,J., Cadkova,Z., Valek,P., Kudrnacova,M. (2011) Competition for minerals (ZE, Mn, Fe, Cu) and Cd between sheep tapeworm (Moniezia expansa) and its definitive host sheep (Ovis aries). Helminthologia, 48, 4:237-243.
12. Jankovskva,I., Vadlejch,J., Szakova,J., Miholova,D., Kunc,P., Knizkova,I., Langrova,I. (2010a) Experimental studies on lead accumulation in the cestode Moniezia expansa (cestoda : Anoplocephalidae) and its final host (Ovis aries). Ecotoxicol. 19, 928-932.
13. Jankovskva,I., Vadlejch,J., Szakova,J., Miholova,D., Kunc,P., Knizkova,I., Cadkova,Z.,. Langrova,I. (2010b) Experimental studies on Cadmium accumulation in the cestode Moniezia expansa (cestoda : Anoplocephalidae) and its final host (Ovis aries). Experiment. Parasitol., 126 :130-134.
14. Kay, J.E., Leventhal, B.G. and Cooper, H.L. (1969) Effects of inhibition of rRNA synthesis on the stimulation of lymphocytes by phyto-haemogglutinin. Exp. Cell. Res. 54: 94-100.
15. Lafferty, K.D. (1997) Environmental parasitology: What can parasites tell us about human impacts on the environment? Parasitol.Today, 13:251-255.
16. Ma, L. (1963) Trace elements and polyphenol oxidase in Clonorchis sinensis. J. Parasitol. 49(2): 197-203.
17. Mathur, S.C. and Pandey, B.P. (1969) Rallietina cesticillus and R. tetragona infections in chicks reared on normal and deficient feeds- an experimental study. Indian J. Anim. Sci. 39: 115-134.
18. Nadakal, A.M. and Nair, K.V. (1987) A comparative study on the mineral composition of the poultry cestode Raillietina tetrarona Molin, 1858 and certain tissues of its host. Proc. Indian Acad. Sci. (Anim. Sci.) 91(2): 153-158.
19. Sharma , R.K., Agarwal, M., Agarwal, S.B. (2010). Physiological, biochemical, and growth responses of lady’s finger( Abelmoschus esculentus L) plants are affected by Cd contaminated soil. Bull. Environ. Contam. Toxicol., 84 :765-770.
20. Singh, B.B.; Singh, K.S.; Ghosal, A.K. and Dwarkanath, P.K. (1978). Inorganic calcium, magnesium and phosphorus in Thysaniezia gigardi. Indian J Parasitol. 2(1): 37-38.
21. Smyth, J.D. (1969) The physiology of cestodes. 1st edition. Oliver and Boyd. Edinburgh.
22. Sures, B. (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends parasitol; 20: 170-177.
23. Von Brand, T. (1973) Biochemistry of parasites. 2nd Edition. Academic Press. N.Y.
24. Von Brand, T. (1979) Biochemistry and physiology of endoparasites. Elsevier North Holland Biomedical Press: Amsterdam.