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DOCUMENTING THE ABUNDANCE AND DIVERSITY OF PROTOZOA IN LEAF LITTER MICROCOSMS USING FOLDSCOPE-THE PAPER MICROSCOPE

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Abstract

Leaf litter provides an excellent source of nutrient for protozoans in freshwater ecosystems and these organisms could be sensitive to changes in seasonality, quantity, diversity and quality of litter. The present study investigated the effect of leaf litter on freshwater protozoan communities from water samples collected from Irumbakkulam, a freshwater pond. Microcosms were set up with leaves of six different plants associated with the pond. Protozoan abundance and diversity was observed for six weeks at weekly intervals using foldscope, an ultra-affordable paper microscope. Interestingly, irrespective of the seasons, the highest number of protozoa was observed in *Mangifera indica* with a value of 1.52×10^2 cells ml^{-1} during the period of study. This was followed by *Gliricidium sepium* with a value of 63 cells ml^{-1} . The lowest was observed in *Mikania micrantha* with 20.4 cells ml^{-1} . The highest diversity was observed in microcosm with bryophyte *Bryum sp.* with a Shannon Weiner index value of 1.79. Protozoans like *Paramecium*, *Arcella*, *Vorticella*, *Colpidium*, *Euplotes*, *Cryptodifflugia*, *Trinema*, *Quadrullella*, *Centropyxis*, *Coleps* and *Heliozoa* were observed. The relationship between litter quality, abundance and diversity is discussed.

Keywords: Foldscope, Leaf litter, Freshwater Protozoa, Irumbakkulam

Introduction

Free-living protozoa are eukaryotic, unicellular, animal-like microbes which are heterogeneous and cosmopolitan in distribution. They thrive in all aquatic and damp environments from open water of oceans, lakes, tree holes, phytotelmata of pitcher plants, sewage and decaying vegetation to anoxic sediments (Finlay, 2002, Cleven, 2004, Plebani et al., 2015). They are characteristically phagotrophic, especially on bacteria, unicellular algae and other protists. Their phagotrophy reinforces their ecological importance in microbial food webs (Azam et al., 1993; Sherr and Sherr, 2002). Although less than 100000 species of protozoans are documented they are virtually ignored within the area of biodiversity conservation, mainly due to their cosmopolitan distribution and fast successional stages (Wey et al., 2009).

In ponds protozoans occupy the lower mid-trophic levels where they consume bacteria and are important prey for larger invertebrates (Xu et al., 2008). Coarse particulate organic matter i.e. plant litter provided by the aquatic plants and trees in the vicinity of ponds is the primary source of energy and carbon for aquatic communities (Webster and Meyer, 1997). Plant litter breakdown is a fundamental ecological process that affects nutrient cycling, nutrient availability, organic matter production and consequently the ecosystem structure in aquatic habitats (Xiong and Nilsson, 1999). Aquatic fungi, bacteria and invertebrate shredders decompose this organic matter and incorporate into aquatic food webs which may be fed by the protozoans and hence transferred to higher trophic levels (Heiber and Gessner, 2002). As these organisms can be sensitive to the changes in litter seasonality, quantity, diversity and quality of organic matter entering the pond from the vegetation associated with pond can thus potentially affect both community structure and function of aquatic ecosystems (Laitung et al., 2002; Barlocher and Graca, 2002). The present

study is an attempt to investigate the relationship between quality and diversity of leaf litter and protozoan communities using microcosms. In the present study an attempt is made to observe the protozoa through the foldscope and photographs taken for further identification.

Methodology

Collection of samples

A small isolated pond, Irumbakkulam in Aluva, Kerala (Lat 10^o.217', Long 76^o.217') was selected for the study from period October to November, 2018. Water samples were collected during the morning hours between 7-8 a.m. using plankton net of 20 microns mesh size. The residue were collected in wide mouthed bottles and transported to the laboratory for further analysis. The vegetation surrounding the pond was noted and six plants associated with the pond were selected for setting up of experimental microcosms. The plants selected were *Mikania micrantha*, *Mangifera indica*, *Alternanthera reineckii*, *Alternanthera philoxeroides*, *Gliricidium sepium* and *Bryum* sp. (Figure 1). Microcosms were set up in duplicates with pond water and leaves and incubated for six weeks. Changes in protozoan abundance and diversity in each experimental microcosms was noted weekly. Leaf quality was analysed using standard techniques. Significant variations in protozoan abundance was calculated using statistical tools like ANOVA-one way and diversity was calculated based on Shannon Weiner diversity index using Primer 7 software.

Observation using foldscope

The foldscope was assembled as per instructions in the foldscope manual (Figure 2). The magnification of the microscope is 140 x and the resolving power is 2 μ . The samples were mounted temporarily on the glass slide using coverslip and fixed with cellophane tape. A smartphone (Samsung J6) with

13.5 pixel camera was used to capture the photographs of protozoa. The taxonomic identification was done based on Patterson (2003).

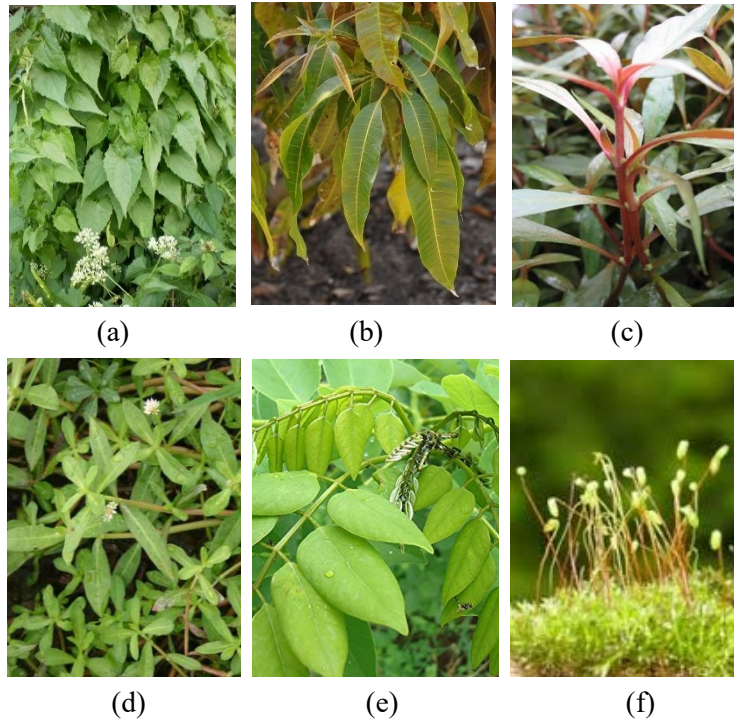


Figure 1. Leaves of plants used for setting up microcosms
(a) *Mikania micrantha* (b) *Mangifera indica*
(c) *Alternanthera reineckii* (d) *Alternanthera philoxeroides*
(e) *Gliricidium sepium* (f) *Bryum* sp.



Figure 2. Foldscope - the paper microscope used for the study

Results

Protozoan abundance and diversity

Protozoan abundance and diversity was observed and recorded in the representative filtered water sample. On an average the protozoan abundance in the pond water sample was 1.33×10^2 cells ml^{-1} . The various protozoans that were observed were testaceans like *Arcella*, *Trinema*, *Assulina*, *Cryptodiffugia*, *Quadrulella*, *Centropyxis*; ciliates like *Paramecium*, *Colpodium*, *Euplotes*; flagellates like *Euglena*; and *Vorticella*, a stalked ciliate and other unidentified protozoa. Interestingly in the microcosms, irrespective of the seasons, the highest number of protozoa was observed in *M.indica* with a value of 1.52×10^2 cells ml^{-1} followed by *G.sepium* with a value of 63 cells ml^{-1} . The lowest was observed in *M.micrantha* with 20.4 cells ml^{-1} (Figure 3). Statistical analysis showed significant variations in weekly protozoan abundance (P value - 0.003). The protozoan abundance in *M.indica* had significant variation with *M.micrantha* (P value-0.025).

On an average, the maximum diversity of protozoans was observed in *Bryum* sp. with a diversity index of 1.79 and the least in *M.micrantha* with the diversity index of 1.09 (Figure 4). The diverse protozoans of the *Bryum* sp. include ciliates like *Paramecium*, *Colpodium*, *Coleps*, *Euplotes* and testaceans like *Arcella*, *Trinema*, *Cryptodiffugia*, *Quadrulella*, *Centropyxis* and *Heliozoa* (Figure 5).

Leaf litter quality analysis has revealed increased carbohydrate and protein content in the leaves of *M.indica* with values of 17.48 and 4.03 respectively and *G.sepium* with respective values of 41.35 and 4.36. The lowest amount of the same was noted in *M.micrantha* with values of 9.97 and 1.64 respectively.

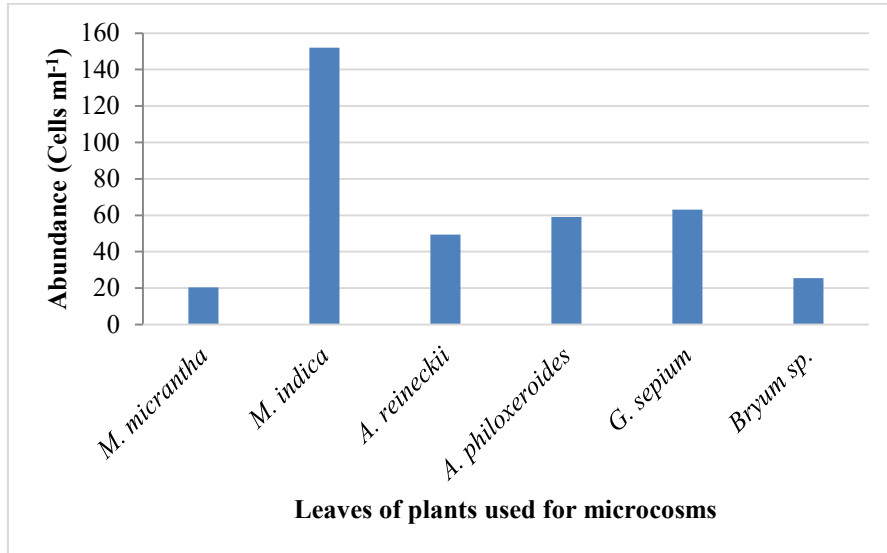


Figure 3. Variation in the abundance of protozoa in leaf litter microcosms

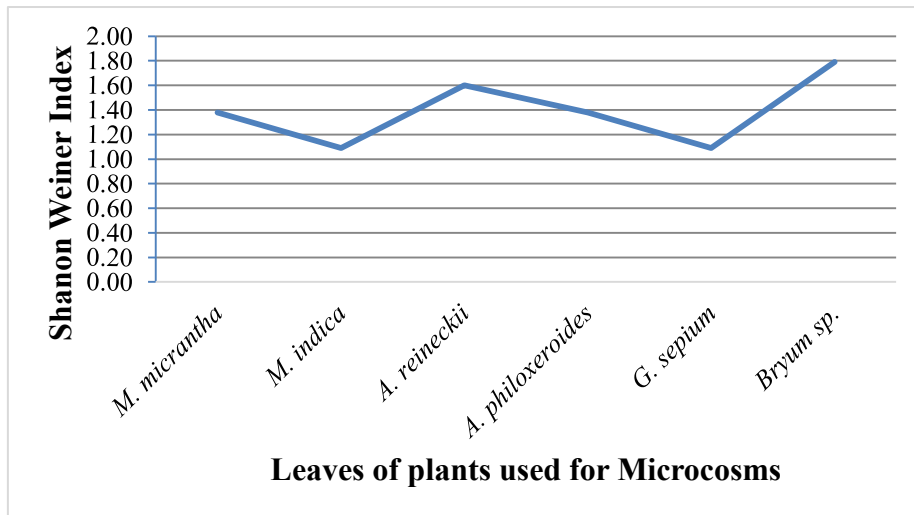


Figure 4. Variation in Shannon Weiner diversity index of protozoan populations in leaf litter microcosms

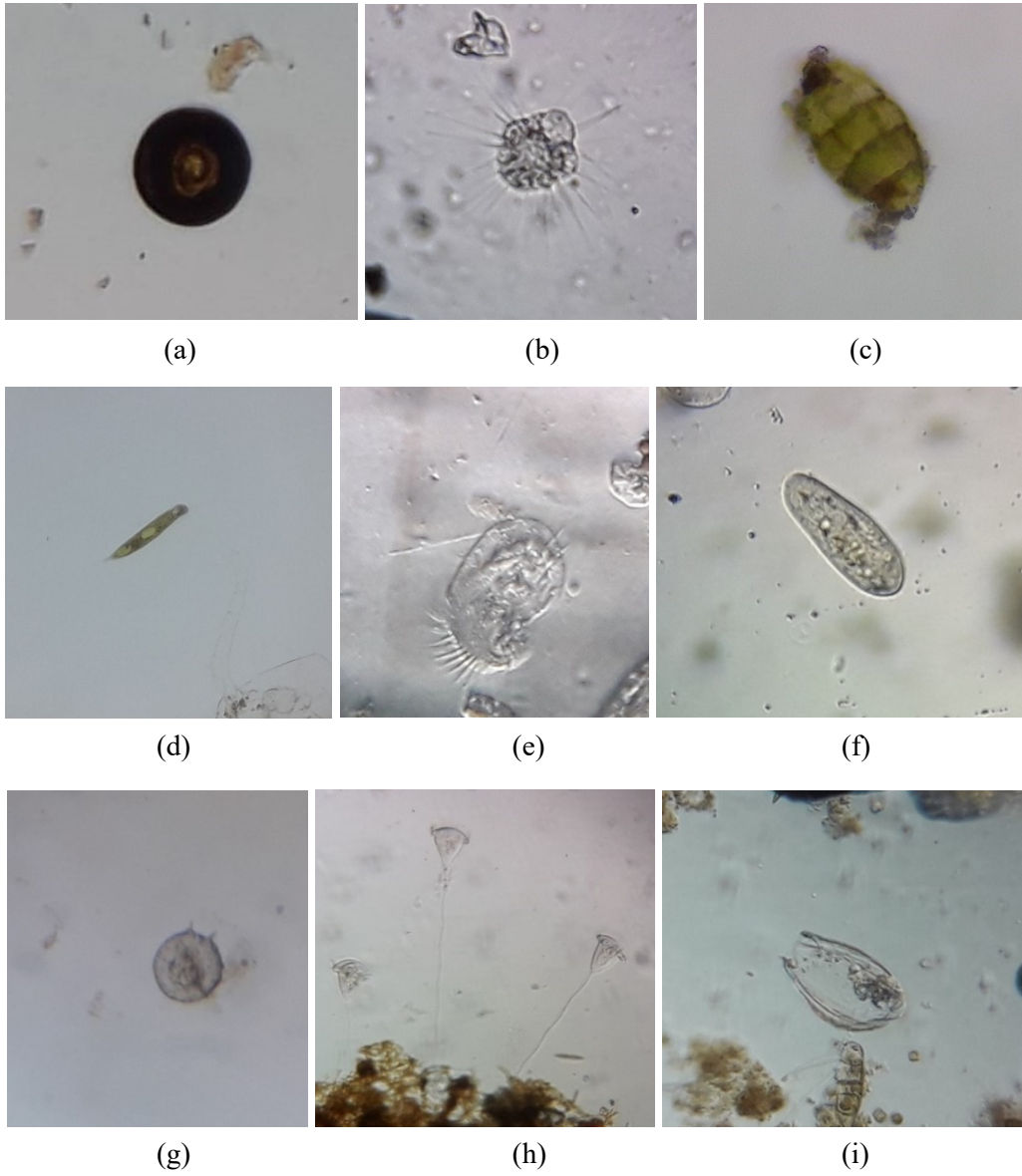


Figure 5. Various protozoa observed under foldscope (a) *Arcella* (b) *Heliozoa* (c) *Quadrelulla* (d) *Euglena* (e) *Euplotes* (f) *Paramecium* (g) *Centropyxis* (h) *Vorticella* (i) *Cryptodiffugia*

Discussion

Ponds are freshwater habitats which may be temporary or permanent that provide a niche for a large number of microorganisms, invertebrates and vertebrates. The pond selected for the present study is a permanent one and showed a protozoan abundance of 1.33×10^2 cells ml^{-1} . Also the pond supports a rich protozoan population like *Arcella*, *Trinema*, *Quadrullella*, *Centropyxis*, *Paramecium*, *Euglena*, *Euplotes*, *Vorticella*, *Assulina*, *Colpodium*, *Cryptodiffugia* and other unidentified protozoa. Protozoans chiefly observed in fresh water environments are rhizopods and ciliates (Ahmad and Sharma, 2009; Radhakrishnan and Jayaprakas, 2015, Chitra, 2014). Though they are widely distributed the composition of the protozoan community may be influenced by the kind of particulate organic matter reaching the aquatic habitat (Nagata and Kirchman, 1992). These protozoa may play a critical role in community organisation by mediating therecycling of nutrients essential for microbial growth and also for micro-ecosystem (Radhakrishnan and Jayaprakas, 2015).

The selected six plants were the dominant plants found associated with the pond. Thus the leaf litter falling into the pond would have a vital role in influencing the composition of protozoan populations. Leaf litter is a common form of particulate organic matter and is a major source of energy and nutrients for increasing the productivity of the basal trophic levels in many ecosystems (Abelho, 2001). In the present study using leaf litter microcosms the highest number of protozoa was observed in *Mangifera indica* with a value of 69.46 cells ml^{-1} followed by *Gliricidia sepium* with a value of 29.6 cells ml^{-1} . This may be attributed to the higher carbohydrate and protein content of leaves of *M. indica*. The lowest was observed in *Mikania micrantha* with 9.2 cells ml^{-1} . The carbohydrate and protein contents of the leaves were lower than the other leaves under study which indicates a low C:N ratio. The allelopathic effects of

M. micrantha on the plants and animals in their vicinity are well documented (Ni et al., 2001; Laurella et al., 2012). From the experiment it was understood that leaf litter had an important role in determining diversity of fresh water protozoans. Plant litter provided by the aquatic plants and trees in the vicinity of ponds is the primary source of energy and carbon for aquatic communities (Gessner et al., 2007; Stoler and Relyea, 2011). Though the highest abundance was observed in microcosm with *M. indica* leaves, the highest diversity was shown in microcosm with *Bryum sp.* Bryophytes form an important abode of a plethora of protozoans as a result of the amphibious nature of these plants. Also these plants provide gradients of abiotic factors offering series of habitat and ample food (Mazei and Tsyganov, 2007; Glime, 2017). From the present study it is clear that though protozoa have a cosmopolitan distribution, exogenous factors such as abiotic factors in conjunction with the quality of available substrate seems to determine the abundance and diversity of protozoan species. From the above study, it was also evident that, Foldscope, the newly invented ultra-affordable paper microscope can be efficiently used to monitor microorganisms.

Conclusion

Protozoans occupy the lower trophic levels of larger water bodies as they are the chief predators of bacteria and they in turn serve as an important prey for larger invertebrates. Thus they form an important component of food chain in water and also play a vital role in determining the structure and function of an aquatic ecosystem. The study showed that the quality and type of leaf litter reaching an aquatic ecosystem could affect the protozoan population dynamics, and this indicates the need of rich and diverse vegetation in and around aquatic habitat. Further studies on the effect of leaf litter on protozoan communities are required to understand the ecosystem functioning and its response to environmental changes.

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References

- Abelho M (2001) From litter fall to breakdown in streams: A review. *The Scientific World*. 1: 656-680
- Ahmad T and Sharma A K (2009) A study of fresh water protozoans with special reference to their abundance and ecology. *Journal of Applied and Natural Science*. 1(2):166-169
- Azam F, Fenchel T, Field J G, Gray J S, Meyer-Reil L A, Thingstad F (1983) The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*. 10: 257-263
- Chitra J (2014) Fauna in freshwater habitats in South Dum Dum Municipality, North 24 Parganas, West Bengal. *Journal of Academia and Industrial Research (JAIR)*. 3 (3).
- Cleven E (2004) Seasonal and spatial distribution of ciliates in the sandy hyporheic zone of a lowland stream. *European Journal of Protistology*. 40: 71-84.
- Finlay B J (2002) Global dispersal of free-living microbial eukaryote species. *Science*. 296: 1061-1063.
- Gessner M V, Gulis K, Kuehn E, Chauvet and Suberkropp K (2007). Fungal decomposers of plant litter in aquatic ecosystems. *Environmental and Microbial Relationships*. 4: 301-324.
- Glime J M (2017) Protozoa Ecology. In: Glime, J. M. *Bryophyte Ecology*. Volume 2. *Bryological Interaction*. Ebook 2-6-1 sponsored by Michigan Technological University and the International Association of Bryologists.
- Hieber, M. and Gessner, MO (2002): Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. – *Ecology* 83: 1026–1038.

- Laurella L C, Frank FM, Sarquiz A, Alonso M R, Giberti G, Cavallaro L, Catalan C A, Cazorla S I, Malchiodi E, Martino V S, and Sulsen V P (2012) In Vitro Evaluation of Antiprotozoal and Antiviral Activities of Extracts from Argentinean *Mikania* Species. The Scientific World Journal. 1-6.
- Mazei Y A and Tsyganov A N (2007) Species composition, spatial distribution and seasonal dynamics of testate amoebae community in a *Sphagnum* bog (Middle Volga region, Russia). Protistology.5 (2/3): 156-206.
- Nagata T and Kirchman D L (1992) Release of DOM by heterotrophic protozoa: implications for microbial food webs. Archives in Hydrobiology BeihErgebn Limnology. 35: 99-109
- Ni G, Li F, Chen B, Song L and Peng S (2007). Allelopathic plants 21. *Mikania micrantha* H.B.K. Allelopathy Journal.19(2): 287-295
- Plebani M, Fussmann K E Hansen D M, O'Gorman E J, Stewart R I A, Woodward G, et al.(2015) Substratum-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams. Freshwater Biology 60:1561-1570.
- Radhakrishnan R and Jayaprakas V (2015) Free living protozoans as bio indicators in Vembanad lake, Kerala, India, an important Ramsar site. International journal of fisheries and aquatic studies. 2(3):192-197.14
- Sherr EB and Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek. 81: 293-3015.
- Stoler A and Relyea R (2011) Living in the litter: the influence of tree litter on wetland communities. Oikos.120: 862-872.
- Wey JK, NorfH, Arndt H, Weitere M (2009). Role of dispersal in shaping communities of ciliates and heterotrophic flagellates within riverine biofilms. Limnology and Oceanography. 54: 1615-1626.
- Xiong S and Nilsson C (1999) The effects of plant litter on vegetation: a meta-analysis. Journal of Ecology.87:984-994
- Xu H, Song W, Warren A, Khaled A, Al-rasheid S.; Saleh A et al.,(2008) Planktonic protist communities in a semi-enclosed mariculture pond: structural variation and correlation with environmental conditions. Journal of the Marine Biological Association of the UK. 88: 1353-1362

EFFECT OF METAL SUBSTITUTION ON COBALT FERRITE $Co_{0.5}M_{0.5}Fe_2O_4$ ($M=Zn, Ni$) NANOPARTICLES

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Abstract

Ferrite nanoparticles exhibit unique chemical, structural and mechanical properties and have a variety of promising technological applications in high density recording devices, ferrofluids, high frequency devices and magnetic refrigerators. Cobalt ferrite is well-known hard magnetic material with relatively high coercivity and saturation magnetization. The structural and magnetic properties of cobalt ferrite could be modified by the substitution of other transition metals like Zn and Ni. The ferrites having the general formula $CoFe_2O_4$ and $Co_{0.5}M_{0.5}Fe_2O_4$ ($M=Zn, Ni$) were synthesized by sol-gel process. The samples were characterized by using X-ray Diffraction (XRD) technique, Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). The results revealed the formation of spinel structure in all the samples. In $Co_{0.5}Zn_{0.5}Fe_2O_4$ sample, Zn^{2+} substitute for Co^{2+} , resulting in an increase in the lattice parameter. The substitution of Co^{2+} ions by Ni^{2+} ions leads to a decrease in lattice parameter in the case of $Co_{0.5}Zn_{0.5}Fe_2O_4$ sample. The single-phase cubic spinel structure of all the three samples were confirmed by XRD. The average crystallite size of the nano crystallites were estimated by determining the full width at half maximum (FWHM) of all the peaks in the XRD pattern. FTIR spectrum confirmed the formation of spinel structure. The magnetic property of the three samples is investigated with a vibrating sample magnetometer at room temperature. The analysis revealed ferromagnetic behavior in all the samples.

Keywords: ferrites, metal substitution, lattice parameter, magnetic properties

Introduction

Nanoscience and nanotechnology pertain to the synthesis, characterization and utilization of nanostructured materials which are characterized by at least one dimension in the nanometer range. Nanotechnology has the potential to lead to breakthrough materials and devices that will change our lives immensely for the better.

Polycrystalline ferrites are the most widely used magnetic materials that exhibit interesting magnetic, electrical and optical properties (Jacob et al., 2012; Jacob et al., 2011; Gopalan et al., 2009). Ferrites with spinel structure often called “spinel” belong to the group of strategic materials which are used in the wide area of modern technology. Magnetic properties of cobalt ferrite nanoparticles are strongly dependent on their chemical compositions and substitutions. The properties of ferrites can be changed dramatically by the addition of small amount of metal ions. For example, Zhang et al., 2000 showed that Ni addition into Co ferrite thin films suppressed the grain formation, resulting in the reduction of surface roughness. Ni²⁺ ions were added to cobalt ferrite to improve the magnetic recording properties of cobalt ferrite films, which are promising for high-density perpendicular recording media.

Also, by substitution of Zn²⁺/Ni²⁺ cation for Co²⁺ cation in the Co-ferrite structure, the structural and magnetic properties can be modified. In the present study synthesis of metal substituted cobalt ferrite nanoparticles and their characterization using XRD, TEM and FTIR techniques are discussed.

Materials and Methods

Synthesis

In this study, the sol-gel method was adopted to synthesize CoFe_2O_4 and $\text{Co}_{0.5}\text{M}_{0.5}\text{Fe}_2\text{O}_4$ (M=Zn, Ni) nanoparticles. The sol-gel method is one of the solution-phase chemical methods which can provide the means of producing high purity crystalline nanoscale materials of a particular size. The sol-gel technique is probably most effective method for the synthesis of homogeneous nano-sized particles (Xavier et al., 2013). This process offers the possibility of a generalised approach to the production of both single and complex oxide nanoparticles. This technique involves hydrolysis and condensation reactions of metal precursors, such as salts or alkoxides, leading to the formation of three-dimensional inorganic networks.

Metal nitrates in the required stoichiometric ratio were dissolved in minimum amount of ethylene glycol at room temperature and the sol was heated at 60°C , to obtain a wet gel. Further heating of the gel at higher temperatures led to self-ignition. The obtained dry powder is ground well using agate mortar to form ultrafine particles of cobalt ferrite. All the three samples in the series were prepared by following similar procedure. To maintain purity and uniform size distribution the samples were sintered at 500°C for 2 hours. These samples named as CF (CoFe_2O_4), NCF and ZCF ($\text{Co}_{0.5}\text{M}_{0.5}\text{Fe}_2\text{O}_4$, M=Zn, Ni) were subjected to further studies.

A. XRD

The structure and crystallite size were determined from the X-ray diffraction data. XRD data were taken at room temperature using CuK_α ($\lambda=1.5406 \text{ \AA}$) radiation. The particle size, lattice constant (a) and X-ray density (ρ_x) were

calculated using the standard relations (Thankachan et al., 2013). The lattice parameter a was computed using the equation

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

where d_{hkl} is the spacing between the planes and (hkl) are the miller indices of the corresponding plane. The most widely used method for calculating the crystallite size is from the FWHM of a diffraction peak by Scherrer formula

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where D is the crystallite size, K is the shape factor ($K=1$), λ is the wave length of the X- radiation, β is the angular width (in radians) which is equal to FWHM. The angle θ for maximum intensity and full width at half maximum β_{abs} were determined from the broadening of the prominent peaks in the XRD pattern. The theoretical (X-ray) density was calculated using the following equation.

$$\rho_x = \frac{8M}{Na^3}$$

where M is the molecular weight (gm) of the sample, N is the Avogadro number (per mol) and a is the lattice parameter (\AA).

B. FTIR

IR spectra of the investigated nano ferrite samples were recorded using FTIR spectrometer. FTIR spectra were recorded for the dried samples of ferrites using Thermo Nicolet, Avatar 370 spectrometer in the wave number range 1500 cm^{-1} to 400 cm^{-1} . The powder samples are mixed with Potassium Bromide (Kbr) matrix and transmittance spectra were recorded. From the characteristic

absorption bands, different functional groups present in the composite can be identified. Hence, IR spectroscopy is very useful in material characterization.

Characterization

The cobalt ferrite samples were characterized by using X-ray powder diffract meter (XRD, Bruker AXS D8 Advance) using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 35 mA.

Results and Discussion

Structural analysis

A. X-ray diffraction analysis

Nanocrystalline cobalt ferrite as well as Zn and Ni substituted cobalt ferrites showed a cubic spinel structure and the XRD patterns are shown in figure 1. The crystallite sizes of the nano crystalline ferrites were calculated by using Scherrer formula. The crystallite size remains within the range 11-14 nm for the studied compositions. The lattice constant, a and X-ray density, ρ_x are tabulated in Table 1. These values are in agreement with the reported values (Thankachan et al., 2013; Xavier et al., 2013).

The X-ray diffraction pattern of NCF reveals that the lattice parameter decreases with the decrease in cobalt concentration when compared to that of CF. This can be explained by the fact that the ionic radius of Co²⁺ (0.78 Å) is larger than that of Ni²⁺ (0.69 Å). For ZCF, the lattice parameter a , increases with the increase in Zn content, confirming that the larger Zn cations substituted for smaller Co cations in the Co ferrite structure.

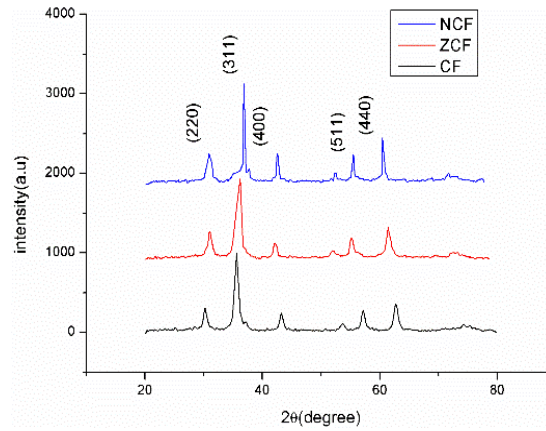


Figure 1. XRD Patterns of CF, NCF and ZCF

Table 1. Crystallite size (D), lattice constant (a) and X-ray density (ρ_x) of CF, NCF and ZCF

Sample	$D(\text{nm})$	$a (\text{\AA})$	$\rho_x(\text{gm/cm}^3)$
CF	14.26	8.36	5.32
NCF	14.94	8.34	5.36
ZCF	11.11	8.37	5.37

B. FTIR spectral measurements

Figure 2 shows the FTIR spectra of the studied samples. All the spectra exhibit two intense bands between 587 cm^{-1} and 400 cm^{-1} belonging to the stretching vibration modes associated to the metal-oxygen absorption bands in the crystalline lattice of Zn and Ni substituted cobalt ferrites. They are characteristically pronounced for all spinel structures and for ferrites in particular. This occurs because the stretching vibration bands related to metal in the octahedral and tetrahedral sites are in this region. In summary, FTIR absorption spectroscopy allows identifying the spinel structure and confirms the XRD structural characterization.

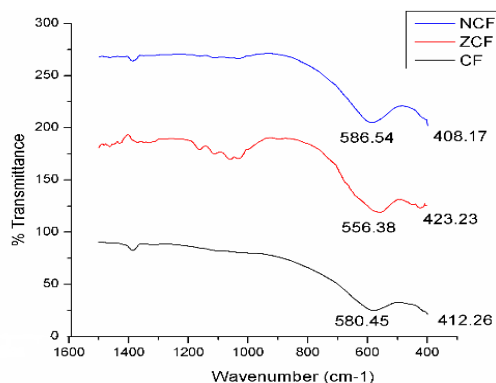


Figure 2. FTIR spectrum of CF, NCF and ZCF

C. TEM analysis

The Transmission Electron Microscope image of the $\text{Co}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ (ZCF) sample is shown in figure 3. Most of the nanoparticles are almost spherical in shape; however, a slight agglomeration is noticed. The size of more than 200 nanoparticles is determined from different images of the same sample using image J software and size distribution histogram is drawn. From figure 4, it can be seen that particles have narrow size distribution. The most probable diameter of the particles was determined as 12.34 nm.

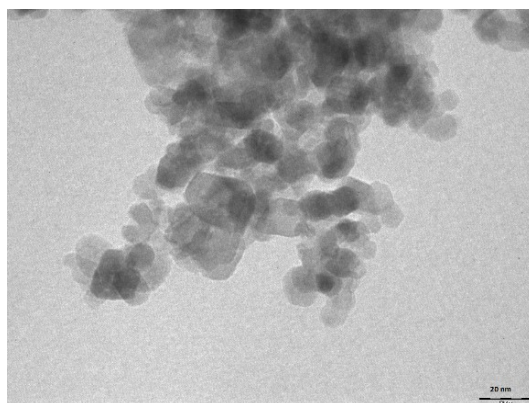


Figure 3. TEM image of ZCF

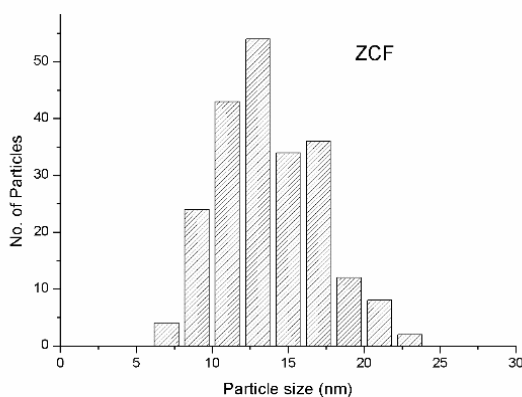


Figure 4. Size distribution of ZCF nanoparticles

D. Magnetic Characterization

The magnetic properties are investigated with a vibrating sample magnetometer (Lakeshore VSM 7410) at room temperature. The figures 5(a), (b), and (c) show the field dependent magnetization (M-H) curves for the samples at room temperature under an applied field of ± 15 kOe. The values of hysteresis measurement of coercive field (H_c), saturation magnetization (M_s) and remnant magnetization (M_r) of the samples are given in Table 2. Cobalt ferrite (CF) sample exhibit hysteresis loops of typical magnetic behaviour, indicating the presence of ordered magnetic structure in the spinel system. They have high coercivity and moderate saturation magnetization. High coercivity of CF confirms that it is a hard magnetic material. Hence cobalt ferrite nanoparticles are promising materials for high density recording applications. The substitution of nickel ions in cobalt ferrite nanoparticles decreased the value of saturation magnetization (M_s) and coercivity (H_c). This may be caused by the substitution of Ni^{2+} ions by Co^{2+} ions on octahedral sites. The magnetic moment (μ) per ion for Co^{2+} ions ($3\mu_B$) is larger than that for Ni^{2+} ions ($2\mu_B$). Therefore the decreasing concentration of Co^{2+} ions on octahedral sites may

result in a decrease in magnetic moment and equivalently a collapse in magnetization.

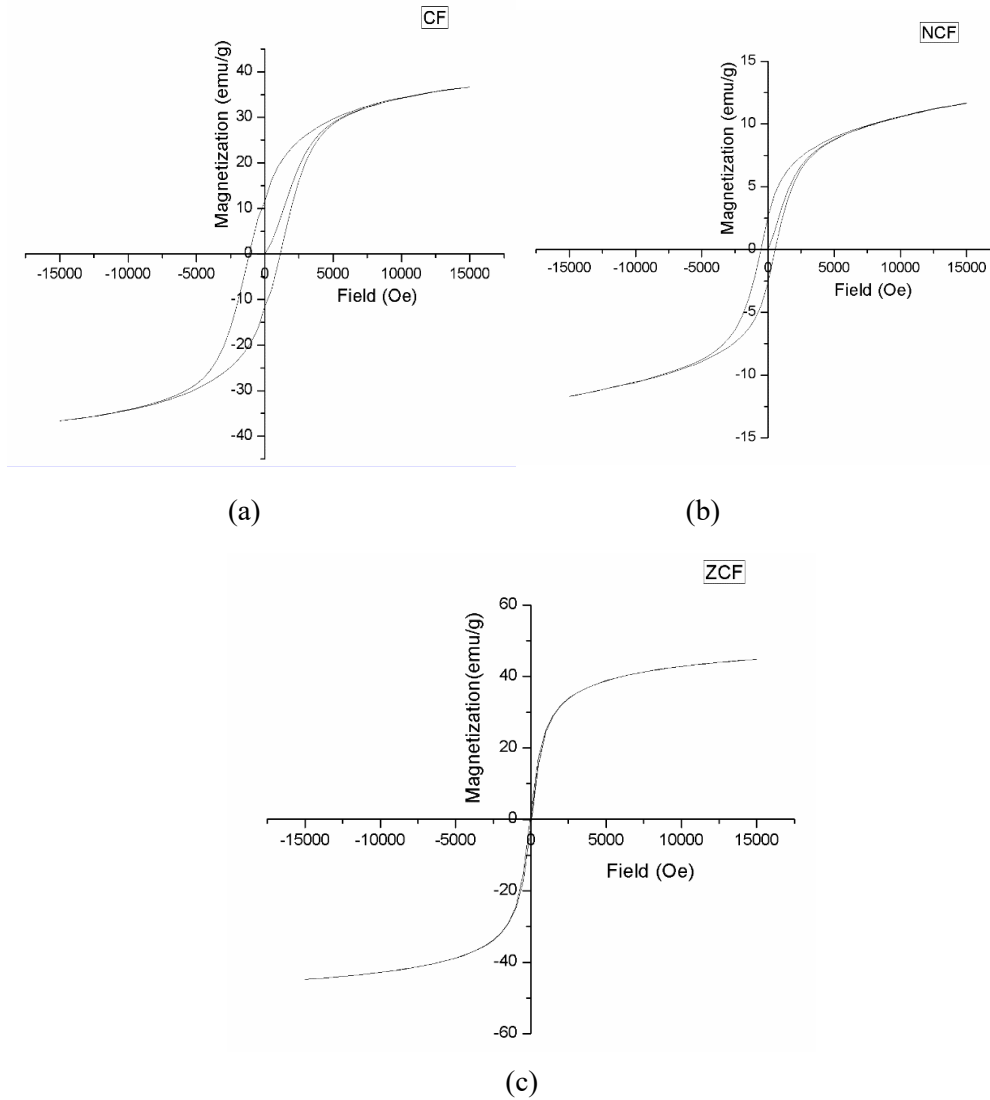


Figure 5. Hysteresis loop of (a) CF, (b) NCF and (c) ZCF

The coercivity of nanoparticles decreases with nickel substitution in cobalt ferrite. The lower magneto-crystalline anisotropy of Ni^{2+} as compared to

that of Co^{2+} ions leads to lower coercivity. From the observed values of magnetization, it is clear that ZCF samples show ferrimagnetic behaviour. The non magnetic Zn^{2+} ions occupy A-sites, replacing magnetic Fe^{3+} ions. This results in decreasing magnetic moment of A-site. At the same time, magnetic moment of B-sites increases due to increase in concentration of magnetic Fe^{3+} and Co^{2+} ions. Thus the net magnetization ($M_B - M_A$) increases (Sebastian et al., 2015). The magnitude of coercive force decreases with the substitution of zinc ions in cobalt ferrite nanoparticles. The low coercive force confirms that the cobalt zinc ferrite is a soft ferrite.

Table 2. Saturation Magnetization, Coercivity, Retentivity and Remnant ratio of the samples

Sample	Saturation Magnetization(M_s) (emu/g)	Coercivity (H_c) (Oe)	Retentivity (M_r) (emu/g)	Remnant ratio (M_r/M_s)
CF	36.643	1139.8	11.522	0.3145
NCF	11.681	537.43	2.619	0.2243
ZCF	44.812	57.499	1.972	0.0440

Conclusions

The results show that sol gel synthesis is a simple and cost-effective method for the preparation of nano ferrite samples. Single phase nanoparticles of CoFe_2O_4 and $\text{Co}_{0.5}\text{M}_{0.5}\text{Fe}_2\text{O}_4$ (M=Zn, Ni) were successfully prepared using this method. The XRD results confirm the spinel structure formation and nano crystallite size in all the samples. The lattice parameter and X-ray density of all the samples are in good agreement with the reported values. Zn^{2+} substituted for Co^{2+} results in an increase in the lattice parameter whereas Ni^{2+} substituted for Co^{2+} results in a decrease in the lattice parameter. The absorption bands in FTIR spectra of all the samples are found in the expected range. Thus, FTIR spectrum

analysis corroborates the XRD results. These results disclosed that the spinel structure of CoFe_2O_4 was not affected by the substitute ions. The TEM analysis reveals that the prepared nanoparticles have spherical morphology, narrow size distribution and are slightly agglomerated. Further studies will reveal the suitability of these materials for magnetic recording and high frequency applications. The magnetization measurement was carried out by VSM. The CF sample exhibit hysteresis loops of ferrimagnetic behaviour, indicating the presence of ordered magnetic structure in the spinel system. The substitution of nickel ions in cobalt ferrite nanoparticles decreased the value of saturation magnetization and coercivity. From the observed values of magnetization, it is clear that ZCF samples show ferrimagnetic behaviour. The variations observed in the magnetic properties with metal substitution in cobalt ferrite nanoparticles reveal the suitability of these material for variety of applications.

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References

- BP Jacob, Thankachan S, Xavier S and E M Mohammed (2011) Effect of Gd^{3+} doping on the structural and magnetic properties of nano crystalline Ni-Cd mixed ferrite, *Physica Scripta*. 84: 045702-08.
- BP Jacob, Xavier S, Thankachan S and E M Mohammed (2012) Dielectric behaviour and ac conductivity of Tb^{3+} doped $\text{Ni}_{0.4}\text{Zn}_{0.6}\text{Fe}_2\text{O}_4$ nanoparticles, *Journal of Alloys and Compounds*. 541: 29-35.
- Gopalan V, I.A.Al-Omari, D Sakthikumar, Yoshida Y, P A Joy and M R Anantharaman (2009) On the structural, magnetic and electrical properties of Sol-gel derived nansized cobalt ferrite. *Journal of Alloys and compounds*. 485: 711-717.

- Thankachan S, B P Jacob, Xavier S and E M Mohammed (2013a) Effect of neodymium substitution in structural and magnetic properties of magnesium ferrite nano particles. *Physica Scripta*. 87: 1-7.
- Thankachan S, B P Jacob, Xavier S and E M Mohammed (2013b) Effect of samarium substitution on structural and magnetic properties of magnesium ferrite nano particles. *Journal of Magnetism and Magnetic Materials*. 348: 140–145.
- Xavier S, Thankachan S, B P Jacob and E M Mohammed (2013) Effect of neodymium substitution on structural and magnetic properties of cobalt ferrite nanoparticles. *Journal of Nanoscience and Molecular Nanotechnology*. 2:7. <http://dx.doi.org/10.4172/2324-8777.1000133>.
- Xavier S, Thankachan S, B P Jacob and E M Mohammed (2013) Effect of Samarium Substitution on the Structural and Magnetic Properties of Nanocrystalline Cobalt Ferrite. *Journal of Nanoscience*. 1-7. <http://dx.doi.org/10.1155/2013/524380>.
- Zhang F, Kitamoto Y, Abe M and Naoe M (2000) Effect of Ni addition into Co ferrite thin films for perpendicular recording media. *Journal of Applied Physics*. 87:6881-6883
- R M Sebastian, Xavier S and E. M. Mohammed (2015) Dielectric Behavior and AC Conductivity of Mg²⁺ Doped Zinc Ferrite Nanoparticles Synthesized by Sol-Gel Technique, *Ferroelectrics*, 481:48-56, 2015, DOI:10.1080/00150193.2015.1049491

STUDY ON THE PREVALENCE OF SIDEROPHORE PRODUCTION IN SELECTED BACTERIAL AND FUNGAL ISOLATES

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Abstract

Iron is crucial for several metabolic processes and microorganisms produce ferric ion specific chelators termed siderophores to promote the rate of Fe^{3+} binding and transport. The current investigation aimed at studying the prevalence of siderophores in some bacterial and fungal species employed CAS agar plate assay as a qualitative methodology for screening siderophore producers. The effect of pH and iron concentration on the siderophore production was examined in Grimm Allen medium. Siderophores were detected in *Aspergillus* and *Penicillium* species with *Aspergillus niger* being the most potent among them. The siderophores produced were of hydroxamate nature. Maximum siderophore production was observed after 9 days of incubation at pH 6 at 1 μ M iron concentration and it remained the same till 10 μ M iron concentration, followed by a decrease at higher levels. This preliminary identification of potential strains further carried out by detailed characterization and purification can result in potent siderophore compounds with variety of applications of academic and industrial interest.

Keywords: Siderophores, *Aspergillus*, CAS assay, Grim Allen medium.

Introduction

Siderophores (from the Greek: “iron carriers”) are defined as relatively low molecular weight, ferric ion specific chelating agents elaborated by bacteria and fungi growing under low iron stress. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cells. Iron is the second most abundant metal after aluminium in the earth’s crust (Howard, 1999). However, its availability to the organisms is very limited due to the rapid oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) state. Ferric ion is highly insoluble under physiological conditions and makes its acquisition by microorganisms and higher organisms a considerable challenge. Microorganisms growing under aerobic conditions need iron for a variety of functions including reduction of oxygen for synthesis of ATP, reduction of ribotide precursors of DNA, for formation of heme, and for other essential purposes. A level of at least one μM iron is needed for optimal growth.

Siderophores, low molecular weight high affinity ferric iron chelators, are synthesized and secreted by many microorganisms in response to iron deprivation (Lankford, 1973). These compounds solubilize and bind iron and transport it back into the microbial cell, usually through specific membrane receptors (Neilands, 1982). Siderophores have been related to virulence mechanism in microorganisms pathogenic to both animals and plants. In establishing an infection, pathogenic microorganisms depend heavily on their ability to use the host complexed iron. To overcome the non-specific defense mechanisms of the host, to circumvent the solubility problem of iron and for its survival within the host, microorganisms produce iron chelators called siderophores (Bullen, 1999). Some siderophores have the ability to remove iron from mammalian iron-binding proteins, and the ability to express these iron

transport systems has been associated with bacterial pathogenicity (Payne, 1988). In addition, they have clinical applications and are possibly important in agriculture (Reichard, 1994). Siderophores can be classified into three main groups depending on the chemical nature of the moieties donating the oxygen ligands for Fe^{3+} (1) hydroxamate (2) catecholate (3) carboxylates. Moreover, several bacteria produce mixed-type siderophores combining different Fe^{3+} ligands in one molecule. With the exception of the carboxylate siderophore rhizoferrin, which is produced by certain zygomycetes, all fungal siderophores identified so far are hydroxamates. Catecholate siderophores such as enterobactin produced by *E.coli* are produced only by certain bacteria. Carboxylates are produced by bacteria (certain *Rhizobium* and *Staphylococcus* strains) and fungi belonging exclusively to mucorales, coordinating iron with carboxyl and hydroxyl groups.

Siderophores have attracted much attention as drugs in facilitating iron mobilization in humans, especially in the treatment of iron diseases and to carry drugs into cells by preparation of conjugates between siderophores and antimicrobial agents. Siderophore biosynthesis is an attractive target in the development of new antibiotics to various diseases (Cole et al., 2003). Desferrioxamine B, produced by *Streptomyces pilosus*, marketed as the mesylate salt under the name Desferal is advocated for removal of excess iron resulting from the supportive therapy for thalassemia (Bergeron et al., 1994), treatment of dialysis encephalopathy (Ackrill et al., 1980). Hydroxamate siderophores present in soil are capable of binding iron and involved in nitrogen fixation by soil bacteria (Powell et al., 1980). Siderophores produced by microorganisms in the rhizosphere can also increase the availability and uptake of iron. Plants such as oats are able to assimilate iron through these microbial siderophores (Kraemer et al., 2006).

The current investigation aimed at studying the prevalence of siderophores in some bacterial and fungal species followed by the fermentative production of siderophores and identification of their chemical nature. Further, the effect of pH and iron on the fermentative production of siderophores was also studied in a selected isolate.

Materials and methods

Microorganisms

Bacterial strains *Salmonella typhimurium* MTCC 98, *Salmonella typhimurium* MTCC 1254, *Bacillus thuringiensis* MTCC86, *Aureobacterium flavescens* MTCC3120, *Escherichia coli* MTCC68, *Staphylococcus aureus* MTCC3103, *Pseudomonas aeruginosa* MTCC2642, *Klebsiella pneumoniae* MTCC2653, *Streptococcus thermophiles* MTCC1938, *Vibrio mereis* MTCC4216, procured from MTCC, Chandigarh. *Pseudomonas* species procured from Poly Clinic, Thrissur, Kerala. Bacterial strains were revived and stock cultures maintained on Nutrient agar slants at 4°C. Fungal cultures *Aspergillus*, *Penicillium* species isolated by air exposure and from soil by serial dilution and maintained on Sabouraud Dextrose Agar at 4°C.

Media and reagents

Chrome azurol sulfonate media (HiMedia Laboratories Pvt. Ltd., Mumbai), Grimm Allen medium (pH- 6.8 adjusted with liquid ammonia), Sodium Succinate Medium (pH-7), Luria Bertani Medium (pH-7) and other reagents of analytical grade were used. All glasswares were cleaned with 3mol/L HCl to remove iron and rinsed in deionized water before use.

Screening of Siderophores -CAS Plate Assay

CAS-blue agar was prepared according to Schwyn and Neilands (Shin et al., 2001) Petridishes were prepared with CAS-blue agar as bottom agar plate. After solidifying, overlays of the appropriate nutrient medium i.e. Luria-Bertani and Sodium succinate agar (*Pseudomonas* species) or Grimm Allen medium were applied over those agar plates in order to be tested for siderophore production. For the qualitative assay, the bacterial/fungal cultures were spot inoculated onto the above petriplates and incubated at 37°C for 24- 48 h. The results were interpreted based on the colour change due to transfer of the ferric ion from its intense blue complex to the siderophore.

Fermentative production of siderophores

Bacterial isolates were grown in 20 ml batches of Luria-Bertani medium (pH-7) and Sodium succinate medium (pH-7, *Pseudomonas* species), incubated for 24-48 h at 37°C with constant shaking at 180-200 rpm on rotator shaking incubator. Fungal strains were grown in 20 ml batches of Grimm Allen medium (pH- 6.8) incubated for 12 days at 28⁰C with constant shaking at 180-200 rpm on rotator shaking incubator. The culture fluids were collected on days, 3, 6, 9, 12 and mycelia were removed by filtering using filter paper (Whatmann No. 1). The pH was recorded and siderophore production was estimated using CAS liquid assay. The percentage of siderophore units was estimated as the proportion of CAS colour shifted using the formula $[(Ar-As)/Ar] * 100$, where Ar is the A630 nm of reference (CAS assay solution+ uninoculated media) and As is the A630 nm of the sample (CAS assay solution + supernatant).

Detection of chemical nature of Siderophores - Tetrazolium Test

To a pinch of tetrazolium salt, were added 1-2 drops of 2N NaOH and 1 ml of culture filtrate. Instant appearance of a deep colour is indicative of hydroxamate siderophores.

Effect of pH on siderophore production

Grimm Allen medium with different pH (4-10) were inoculated with *Aspergillus niger* spore suspension, incubated for 12 days at 28⁰C with constant shaking at 180-200 rpm on rotator shaking incubator. At 3 days of intervals, samples were taken and siderophore production estimated using CAS liquid assay.

Effect of iron concentration on siderophore production

Grimm Allen medium (pH- 6.8) containing iron in increasing amounts i.e. 0-50 μ M was used in this experiment. The fermented broth was harvested at 3 day intervals, and siderophore production quantitated using CAS liquid assay.

Results and discussion

Screening of Siderophores -CAS Plate Assay

CAS agar petriplates inoculated with bacterial species showed no characteristic colour change after 24-48 h of growth. Various bacterial strains like *Salmonella typhi*, *Pseudomonas*, *E. coli* etc have been reported to produce a number of catecholate siderophores. Hence, the results in the present study with regard to the negative siderophore production may be treated as “non-detection” than non-production. Among the fungal strains, growth on CAS-agar petriplates for siderophore detection, showed the following responses: rate of growth, reaction colour and rate of CAS reaction. The results were qualitatively distinct

in the terms of color of CAS reaction, and changes from blue to orange, purple or dark purplish were observed.

After incubating for a period of 24 h in dark at 28⁰ C, growth was observed for both *Aspergillus* and *Penicillium* species. The colour development in CAS plates was shown to increase with incubation time and was recorded till 120 h. A color change from dark blue to purple was observed within 24 h in the bottom agar plates, surrounding *Aspergillus niger* and color change from dark blue to orange yellow was observed around *Aspergillus flavus*. A change in color from dark blue to orange yellow was observed after 48 h around *Penicillium* species and color change in the CAS blue agar was observed after longer incubation periods. The intensity of orange zone varied indicating different amounts of siderophore production being produced. *Aspergillus niger* showed stronger colour reaction compared to the other isolates (Figure 1, Table 1).

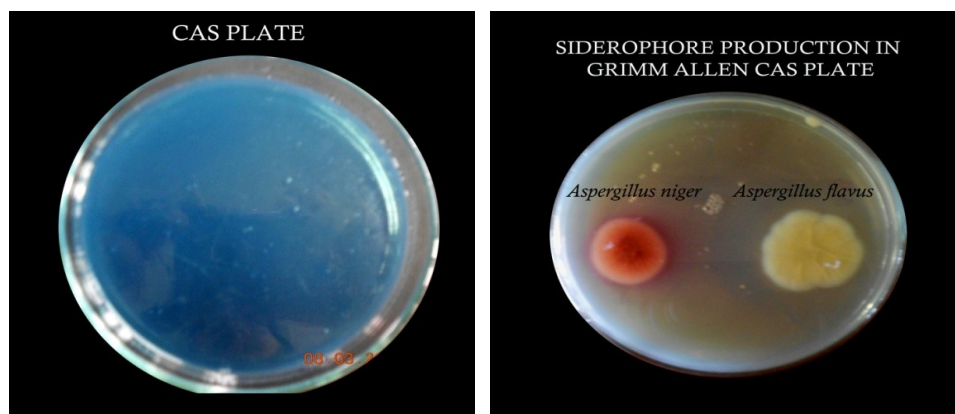


Figure 1 CAS Plate assay for siderophore production

Fermentative production of siderophores

The siderophores produced over a period of 3-12 days were quantified using CAS liquid assay. The siderophore production over the fermentation period showed a characteristic pattern with an increase towards stationary phase of growth and a decline towards the final stages of culture growth in the case of *Aspergillus* and *Penicillium* species (Figure 2).

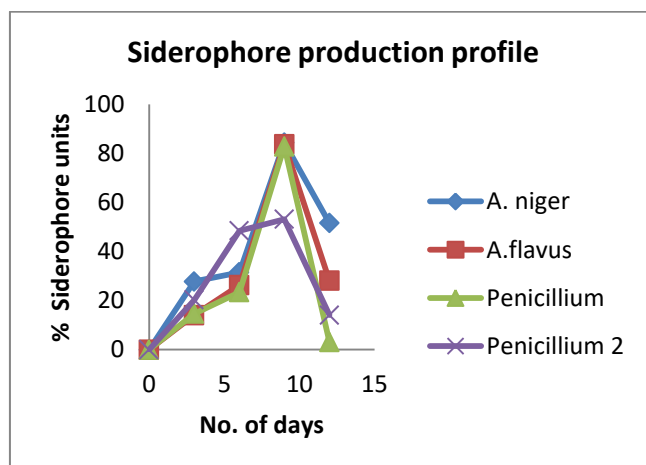


Figure 2 Siderophore production profile

Table 1 Siderophores production by fungal isolates

Microorganism	Day 3 % SU	Day 6 % SU	Day 9 % SU	Day 12 % SU
<i>Aspergillus niger</i>	27.81	31.5	84.37	51.56
<i>Aspergillus flavus</i>	14.06	26.25	83.75	28.12
<i>Penicillium</i>	14.68	23.43	82.81	3.12
<i>Penicillium 2</i>	20.3	48.43	53.12	14.06

Aspergillus niger was thus chosen for further studies to determine the effect of various parameters like pH and iron concentrations on siderophore production.

Detection of chemical nature of siderophores

A hydroxamate specific assay was used to determine the chemical nature of siderophores. An immediate deep red colour formation on the addition of culture fluid from the CAS positive *Aspergillus* species to a pinch of tetrazolium salt and NaOH confirmed the hydroxamate nature of these compounds. The colour formation gradually decreased with incubation period which is in agreement with the siderophore production pattern.

Tetrazolium test is a reliable and widely utilized method pioneered by the German scientist Lakon during the 1939-1958, which can detect compounds which reduce 2, 3, 5 triphenyl tetrazolium chloride or bromide, a colourless chemical into a red coloured compound formazan. This test is extensively used to confirm the hydroxamate nature of siderophores.

Effect of pH on siderophore production

One of the factors which play an important role in the solubility of iron is pH, thereby making its availability to the organism for its growth. The Grim-Allen medium adjusted at different pH was used to evaluate the effect of pH on siderophores production by *Aspergillus niger* over a period of 12 days using CAS liquid assay and the results were tabulated (Table 2). The rate of growth and CAS reaction were affected by different pH (4-8) in the growth medium.

Table 2 Effect of pH on siderophore production by *Aspergillus niger*

pH	Day 3 (% SU)	Day 6 (% SU)	Day 9 (% SU)	Day 12 (% SU)
4	31.25	25	53.12	37.5
5	6.25	62.5	70.31	48.43
6	31.5	57.81	84.37	51.56
7	23.4	29.65	80.62	10.93
8	39.06	7.81	76.5	21.87
9	15.52	26.56	59.37	23.43
10	39.06	46.87	75	41.56

It was seen that there was a steady increase in growth and siderophore production at different till 9th day though further incubation resulted in a decrease in the siderophore yield. The most significant influence was observed with *Aspergillus niger* growing at pH 6.0 which had the highest growth and hydroxamate production. Alternatively, cells grown on pH 4, 5, 7, 8, 9 and 10 had significantly less growth and CAS reaction. In general, the best pH of medium for growth in case of *Aspergillus niger* was 6.0.

Effect of iron concentration on siderophore production

The effect of iron on siderophores production by *Aspergillus niger* over a period of 12 days was quantified with 0-50 μM iron concentrations using CAS liquid assay and the results were tabulated (Table 3). The results in the liquid medium showed that as the concentration of iron increased, rate of siderophores production decreased and growth rate increased. Maximum siderophore production was observed at 1 μM iron concentration and it remained the same till 10 μM iron concentration. A notable decrease in the rate of siderophore production was seen from 10 μM to 50 μM concentration of iron but the growth was maximum at high iron concentration. It has been reported that iron

concentration of 50 μM is high and generally results in excellent growth with only modest yields of siderophores (Neilands, 1984). Thus although cell growth reached a maximal value above 10 μM added Fe (III), siderophores biosynthesis was lowered in this concentration, since cell growth and the siderophores production are inversely proportional responses.

Table 3 Effect of Iron on siderophores production by *Aspergillus niger*

Concentration of Iron (μM)	Yield (% SU)
0	95.16
1	96.31
5	91.78
10	91.15
20	82.41
30	56.29
40	32.20
50	25.71

Conclusions

Siderophores are specific ferric iron binding compounds produced by microorganisms which promote the rate of Fe^{3+} transport. Since iron is crucial for several metabolic processes, microorganisms have developed different mechanisms to overcome this limitation, an important being production of ferric ion specific chelators termed siderophores. The current investigation was aimed at studying the prevalence of siderophores in some bacterial and fungal species. The CAS agar plate assay as a qualitative methodology for evaluating siderophore production as a function of time was used to screen siderophore producers. It was also attempted to evaluate the effect of pH and iron concentrations on siderophore production in liquid medium.

Siderophores were detected in *Aspergillus* and *Penicillium* species with *Aspergillus niger* being the most potent among them. The siderophores produced were of hydroxamate nature. Maximum siderophore production was observed after 9 days of incubation at pH 6 at 1 μ M iron concentration and it remained the same till 10 μ M iron concentration, followed by a decrease at higher levels. This preliminary identification of potential strains further carried out by detailed characterization and purification can result in potent siderophore compounds with variety of applications of academic and industrial interest.

References

- Ackrill P, Raiston A J, Day J P and Hodge K C (1980) Successful removal of aluminum from patients with encephalopathy. *Lancet* 2: 692-693.
- Bergeron R J and Brittenham G M (1994) *The Development of Iron Chelators for Clinical Use*, CRC Press, Boca Raton. 353-371
- Bullen J J and Griffiths E (1999) Iron binding proteins and Host Defense, In: *Iron and infection*. 2nd Edn, John Wiley and Sons Ltd., New York, USA, 327-368.
- Cole S T, Eisenach K D, McMurray D N and Jacobs W R J (2003). *Tuberculosis and the Tubercle Bacillus* ASM Press, Washington DC 341-357
- Howard D H (1999) Acquisition, transport and storage of iron by pathogenic fungi. *Clinical Microbiology Review*. 12: 394-404.
- Kraemer, Stephan M, Crowley, David Kretzschmar and Ruben (2006) Siderophores in Plant Iron Acquisition: Geochemical Aspects *Adv Agron*. 91: 1-46.
- Lankford C E (1973) Bacterial assimilation of iron. *Critical Review in Microbiology*. 2: 273.
- Neilands J B (1982) Iron uptake by fungi: contrast mechanism with internal or external reduction. *Annual Review in Microbiology*. 36: 285.

- Neilands J B (1984) Methodology of siderophores. Siderophores from microorganisms and plants. *Structure and Bonding*.58: 1–24.
- Payne S M (1988) Iron and virulence in the family enterobacteriaceae. *Critical Review in Microbiology*. 16- 81.
- Powell P E, Cline G R, Reid C P P and Szaniszlo P J (1980) Occurrence of hydroxamate siderophore iron chelators in soils. *Nature*. 287: 833-834.
- Reichard P (1994) Bleomycin genotoxicity alteration by glutathione and cytochrome. *Journal of Biological Chemistry*. 268: 8383–8386.

ENDOPARASITIC INFECTIONS OF CAPTIVE MONKEYS IN THE ZOO'S OF KERALA, SOUTH INDIA

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Abstract

This study was undertaken to evaluate the status of parasitic infections in captive monkeys of Kerala using coprological samples. Samples were collected from captive monkeys housed at State Museum and Zoos of Thrissur and Thiruvananthapuram. This is a preliminary parasitic investigation on captive monkeys of Thrissur zoo. Parasites were screened by direct smear, sedimentation, simple floatation and Shaether's sugar floatation method. Of the five species of monkeys; 23 samples were screened, only nine samples (39.1%) were found to be positive. Infection rates were 33% and 50% in Thrissur and Thiruvananthapuram zoo samples respectively. *Ascaris* sp. was the most identified parasite accounting for 77.7 % of infections. *Entamoeba* sp. and *Taenia* sp. infection were restricted to Thrissur zoo samples whereas *Balantidium* cyst infection was found only in Thiruvananthapuram zoo samples.

Keywords: Lion tailed Macaque, Nilgiri Langur, Thiruvananthapuram Zoo, Thrissur Zoo

Introduction

Primates are the highest order of mammals which includes lemurs, monkeys, apes, humans and other similar forms (Mary et al., 2007). They are susceptible to directly transmitted parasitic infections as they often live in close social groups that facilitate their transmission (Stoner et al., 2005). Monkeys act as intermediate host of many parasites which is likely to be transmitted to humans. They are vulnerable to both helminth and protozoan parasites (Munene et al., 1998; Muriuki et al., 1998). Many of the parasites of primates are transmitted orally, either when the monkey ingests contaminated water, foods, or by an intermediate host containing the infective stage of a parasite (Nunn and Altizer, 2006; Chapman et al., 2009).

Parasitic prevalence in captive animals can be used as indicators of population and ecosystem health (Teichroeb et al., 2009). A high rate of infection can result in malnutrition, weakened immunity, movement and feeding. It can also lead to fatal lesions which in turn cause secondary infections, blood loss, tissue damage and death (Toft and Eberhard, 1998; Packer et al., 2003). Effects of parasitism can be augmented when parasites are retransmitted to individuals that are not immunologically sound (Viggers et al., 1993). Confined areas in zoos makes captive animals more prone to different parasitic infections. The health status of the animals in captivity depends on many factors like feeding, maintenance of hygiene in captivity, environmental conditions etc. (Kashid et al., 2002).

In India, systematic investigation of parasitic diseases of wildlife is still in its early stages. There appears to be not many studies on the prevalence of gastro-intestinal parasites in captive monkeys of the zoos of Kerala. The present work is an investigation on the prevalence of gastrointestinal parasites in

captive monkeys of two major zoos in Kerala- State Museum and Zoo, Thrissur and Thiruvananthapuram Zoo, based on coprological samples; as these samples involve non-invasive procedures and provide data to be included in the analysis of ecological relationships between primates and their environment (Eckert et al., 2006; Gillespie, 2006).

Methodology

Faecal samples were collected during July-November 2017 from five species of captive monkeys viz., *Macaca radiata* (Bonnet macaque), *Macaca mulatta* (Rhesus macaque), *Macaca silensis* (lion-tailed macaque), *Semnopithecus* sp. (Hanuman langur) and *Trachypithecus johnii* (Nilgiri langur) housed at State Museum and Zoo, Thrissur (10°31'N, 76°12'E) and Museums and Zoo, Thiruvananthapuram (8°30'N, 76°57'E). Nilgiri langurs and Lion-tailed macaques are endemic to Western Ghats and listed in Schedule I of Indian Wildlife Protection Act (1972). Approximately 3-4 gm of non-desiccated monkey faecal samples were collected from State Museums and Zoos of Thrissur (n = 15) and Thiruvananthapuram (n = 8). A total of (n = 23) faecal matter samples were collected and placed it in 10 ml of 4% formaldehyde. We stored the samples at room temperature.

Direct Smear, Faecal floatation, Shaether's sugar floatation with a specific gravity (SG) of 1.23 to 1.27 and sedimentation technique was used for faecal examination (Wolf et al., 2014; Soulsby, 1982). Parasitic elements (eggs, oocysts and larvae) were counted using a light microscope under ×400 and ×1000 magnification. We identified parasite taxa based on identified characteristics of known stages with the guidelines reported in literature (Schneller and Pantchev, 2008).

Results

We analysed 23 coprological samples from five species of monkeys viz., *Macaca radiata*, *Macaca mulatta*, *Macaca silensis*, *Semnopithecus* sp. and *Trachypithecus johnii* for endoparasites. Only nine samples (39.1%) were found to be positive. In, the Thrissur zoo samples, 33% of the monkey samples were found to be positive for parasitic infections whereas infection rate was 50% in Thiruvananthapuram zoo samples. *Ascaris* sp. was the most identified parasite accounting for 77.7 % of infections. Helminth infections were more prevalent than protozoan infections (Table. 1). *Entamoeba* sp. and *Taenia* sp. infection were restricted to Thrissur zoo samples whereas *Balantidium* cyst infection was found only in Thiruvananthapuram zoo samples.

Table 1. Endoparasitic infection of the captive monkeys from Thrissur State Museum and Zoo and Thiruvananthapuram Museums and Zoo

	State Museum and Zoo, Thrissur			Museums and Zoo, Thiruvananthapuram			
	Rhesus macaque (<i>Macaca mulatta</i>) (*n=9)	Bonnet macaque (<i>Macaca radiata</i>) (*n=3)	Lion-tailed macaque (<i>Macaca silensis</i>) (*n=3)	Host			
	Rhesus macaque (<i>Macaca mulatta</i>) (*n=2)	Hanuman langur (<i>Semnopithecus</i>) (*n=2)	Nilgiri langur (<i>Trachypithecus johnii</i>) (*n=2)	Lion-tailed macaque (<i>Macaca silensis</i>) (*n=2)			
<i>Entamoeba</i> sp.	3(33.33)	-	-	-	-	-	-
<i>Ascaris</i> sp.	3(33.33)	-	2(66.66)	-	-	2(100)	-
<i>Capillaria</i> sp.	-	-	-	-	-	2(100)	-
<i>Balantidium</i> cyst	-	-	-	1(50)	-	-	1(50)
<i>Trichuris</i> sp.	3(33.33)	-	-	-	-	-	-
<i>Taenia</i> sp.	-	-	2(66.66)	-	-	-	-
Infected	3(33.33)	0	2(66.66)	1(50)	-	2(100)	1(50)
Non Infected	6(66.66)	3(100)	1(33.33)	1(50)	2(100)	-	1(50)

*n = no. of samples, values inside brackets indicates percentages

Discussion

Endoparasitic infection studied in the coprological samples of monkeys belonging to five species from two zoos viz., Thrissur and Thiruvananthapuram of Kerala state revealed that 39.1% of the captive monkeys were infected. In the Thiruvananthapuram samples, infection rate was 50%. Earlier investigation on *Macaca radiata* and *Macaca mulatta* of this zoo found the infection rate at 45%. *Strongyloides* sp., and *Balantidium coli* were the common parasites observed by Varadharajan and Pythal (1999) in the Thiruvananthapuram samples, whereas in this study most common helminth infection was with *Ascaris* sp. In the snake samples of Thrissur and Thiruvananthapuram zoo the infection rate was found to be very high at 71.4% and 80% respectively (Akhila et al., 2018).

Studies at Nandan Van Zoo, Chattisgarh, India reported the prevalence of parasitic infection in Rhesus macaques at 60% (Thawait et al., 2014) while Reddy (1992) reported 0% prevalence of endoparasites in Rhesus monkeys from Bangalore. A higher incidence of parasitic infection in monkeys (89.47%) and langurs (66.66%) was noted by Bante (2012). Wongsawad (2009) observed protozoan parasites like *Entamoeba coli*, *Entamoeba histolytica*, *Balantidium coli*, *Isospora* sp. and *Eimeria* sp. from Assamese macaque (*Macaca assamensis*) of Thailand, whereas helminth parasite included *Toxocara* sp., *Oesophogostomum* sp., *Trichuris* sp., *Ascaris* sp. and *Strongyloid* sp. We could not find any published works on the parasitological infection values in the captive monkeys of Thrissur zoo.

Study by Arjun et al. 2015 at Pookode, Wayanad in Bonnet macaque reported *Strongyle* sp., *Stroglyoides* sp. and *Enterobius vermicularis*. Though a common parasite, *Strongyloides* sp. was reported previously in the macaques by

many authors (Varadharajan and Pythal, 1999; Teichroeb et al., 2009; Hussain et al., 2013; Hernandez et al., 2009; Parr et al., 2013; Gunasekera et al., 2012), we could not identify them in our samples. Incidence of parasitic infection in free living Rhesus Macaque in temple premises reported infection rate as 43% with incidence of three parasite species viz., *Strongyle* sp., *Ascaris* sp. and *Eimeria* sp. (Arunachalam et al., 2015). The frequency of low parasitic infection may be due to their feeding habit of leaves of particularly those of medicinal values, like neem and pomegranate leaves in the area which decrease the parasitic egg counts (Parmar et al., 2012). *Strongyloides* sp. and *Trichuris* sp. were the most prevalent endoparasites in samples collected from Western Ghats endemic Nilgiri langurs and lion tailed macaques of fragmented forest habitats in South India (Tiwari et al., 2017; Hussain et al., 2013).

Protozoan parasites such as *Giardia* sp., *Entamoeba* sp. and helminth parasites like *Strongyloides* sp., *Enterobius* sp. and *Trichuris* sp. are zoonotic and are pathogenic to humans (Tailor et al., 2001; Weyher et al., 2006; Murray et al., 2000). Nematode parasites like *Ascaris* sp. and *Capillaria* sp. does not require any intermediate host and they are easily transmissible through contaminated food and water (Smith, 1985). *Giardia lamblia* and *Entamoeba histolytica* are the most common causative agents of dysentery and diarrhoea in humans. Helminth parasites like *Trichuris* sp. and *Taenia* sp. amass easily in the captive soil enclosures and cannot be easily disinfected. Severe helminth parasitic infection can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations and death (Despommier et al., 1995). There are chances of interspecific disease transmission (Daszak et al., 2001) of these parasites to human population as there is frequent contact between monkeys and zoo keepers and other human visitors in zoos.

Conclusion

In group living primate species, the transmission of endoparasites is mostly from individual to individual within the population, so the rate of transfer takes place very rapidly necessitating regular surveillance of these primates. Analysis of endoparasites should also be extended to other captive animals in these zoos, with increasing number of faecal samples to enhance the sensitivity of results.

Acknowledgements

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References

- Akhila S, Sukanya VS, Anto A and Karunakaran S (2018) Prevalence of endoparasites in captive snakes of Kerala, India. *Annals of Parasitology*. 64 (2):129-135.
- Arjun CP, Ravindran R and Anoopkumar T (2015) A Study of Gastrointestinal Parasites in Bonnet Macaque (*Macaca radiata*) of Pookode, Wayanad, Kerala. *Zoos' Print Journal*. 30 (10):7-8.
- Arunachalam K, Senthivel K and Anbarasi P (2015) Endo parasitic infections in free living Rhesus Macaque *Macaca mulatta* of Namakkal, Tamil Nadu, India. *Zoos' Print Journal*. 30 (6):20-21.
- Bante S (2012) Prevalence and efficacy of drugs against helminth parasites in captive wild animals. Thesis, Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur, Madhya Pradesh
- Chapman CA, Sam Hodder and Rothman JM (2009) Host-parasite dynamics: connecting primate field data to theory. In: *Primate parasite ecology: The dynamics and study of host-parasite relationship*. Huffman MA and Chapman CA (ed) Cambridge: Cambridge University Press, pp 463-483.

- Daszak P, Cunningham AA and Hyatt AD (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*. 78:103–116.
- Despommier DD, Gwazda RW and Hotez PJ (1995) *Parasitic Diseases*. Springer-Verlag, New York.
- Eckert KA, Hahn NE, Genz A, Kitchen DM, Stuart MD, Averbek GA, Stromberg BE and Markowitz H (2006) Coprological surveys of *Alouattapigra* at two sites in Belize. *International Journal of Primatology*. 27: 227–238.
- Gillespie TR (2006) Noninvasive assessment of gastro-intestinal parasite infections in free-ranging primates. *International Journal of Primatology*. 27:1129–1143.
- Gunasekera UC, Wickramasinghe S, Wijesinghe G and Rajapakse RPVJ. (2012) Gastrointestinal parasites of captive primates in the national zoological gardens of Sri Lanka. *Taprobanica* 4: 37-41.
- Hernandez AD, Macintosh AJ and Huffman MA (2009) Primate parasite ecology: patterns and predictions from an ongoing study of Japanese macaques. Cambridge University Press 387-401.
- Hussain S, Ram MS, Kumar A, Shivaji S and Umapathy G (2013) Human Presence Increases Parasitic Load in Endangered Lion-Tailed Macaques (*Macacasilenus*) in Its Fragmented Rainforest Habitats in Southern India. *PLoS ONE*. 8(5): e63685. doi:10.1371/journal.pone.0063685
- Kashid KP, Shrikhande GB and Bhojne GR (2002) Incidence of gastrointestinal helminths in captive wild animals at different locations. *Zoos' Print Journal*. 18: 1053-1054.
- Mary TS, Eric JS, Jonathan IB and Doug MB (2007) Primate Origins and Supraordinal Relationships: Morphological evidence. In: Winfried Henke HHC and Ian Tattersall (ed) *Handbook of paleoanthropology. Primate evolution and human origins volume 2*. Springer-Verlag Berlin Heidelberg, New York, pp 831-860.

- Munene E, Otsyula M, Mbaabu DA, Mutahi WT, Muriuki SM and Muchemi GM (1998) Helminth and protozoan gastrointestinal tract parasites in captive and wild trapped African nonhuman primates. *Veterinary Parasitology*. 78: 195-201.
- Muriuki SMK, Murugu RK, Munene E, Karere GM and Chai DC (1998) Some gastro-intestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. *Acta Tropica*. 71: 73-82.
- Murray S, Stem C, Boudreau B and Goodall J (2000) Intestinal parasites of baboons (*Papio cynocephalus anubis*) and chimpanzees (*Pan troglodytes*) in Gombe National Park. *Journal of Zoo Wildlife Medicine*. 31: 176-178.
- Nunn CL and Altizer S (2006) *Infectious diseases in primates-behaviour ecology and evolution*. Oxford University Press, United Kingdom.
- Packer C, Holt RD, Hudson PJ, Lafferty KD and Dobson AP (2003) Keeping the herds healthy and alert: implications of predator control for infectious disease. *Ecology Letters* 6:797–802.
- Parmar SM, Jani RG and Mathakiya RA (2012) Study of parasitic infection in non-human primates of Gujarat state, India. *Veterinary World*. 5: 362-364.
- Parr NA, Linda MF and Kutz SJ (2013) A corpological survey of parasites in white faced capuchins (*Cebuscapuicinus*) from Sector Santa Rosa, ACG, Costa Rica. *Folia Primatologica* 84: 102-114.
- Reddy NRJ, Jagannath MS, Souza PD, Rahman SA and Basavarajappa K (1992) Prevalence of gastrointestinal parasites in wild mammals and captive birds at Bannerghatta National Park, Bangalore. *Indian Journal of Animal Science*. 62(11):1046-1048.
- Schneller P and Pantchev N (2008) *Parasitology in Snakes, Lizards, and Chelonians – A Husbandry Guide*. Frankfurt am Main: edition Chimaira.
- Soulsby EJJ (1982) *Helminths, arthropods and protozoa of domesticated animals*. 7th ed. Bailliere Tindall, London.

- Stoner KE, Gonzalez-Di Pierro AM and Maldonado-Lopez S (2005) Infecciones de parásitos intestinales de primates: implicaciones para la conservación. *Universidad y Ciencia* (Sp. issue II):61–72.
- Taylor LH, Latham SM and Woolhouse ME (2001) Risk factors for human diseases emergence. *Philosophical Transactions of Royal Society B Biological Sciences*. 356: 983-989.
- Teichroeb JA, Kutz SJ, Parker U, Thompson ARC and Sicotte P (2009) Ecology of the gastrointestinal parasites of *Colobus vellerosus* at Boabeng-Fiema, Ghana: possible anthroponotic transmission. *American Journal of Physical Anthropology*. 140: 1-10.
- Thawait VK, Maiti SK and Dixit A A (2014) Prevalence of gastro-intestinal parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhattisgarh. *Veterinary World*. 7(7): 448-451.
- Tiwari S, Reddy DM, Pradheeps M, Sreenivasamurthy GS and Umopathy G (2017) Prevalence and co-occurrence of gastrointestinal parasites in Nilgiri Langur (*Trachypitecus johnii*) of fragmented landscape in Anamalai Hills, Western Ghats, India. *Current Science*. 113(11): 2194-2200.
- Toft JD and Eberhard ML (1998) Parasitic diseases. In: *Nonhuman Primates in Biomedical Research Diseases*. Bennett BT, Abee CR, Henrickson R (eds) San Diego: Academic Press.
- Varadharajan A and Pythal C (1999) A preliminary investigation on the parasites of wild animals at the Zoological Garden, Thiruvananthapuram, Kerala. *Zoos Print Journal*. 1-14,3-12:159-164.
- Viggers KL, Lindenmayer DB and Spratt DM (1993) The importance of disease in reintroduction programs. *Wild Research*. 20: 687–698.
- Weyher AH, Ross C and Semple S (2006) Gastrointestinal parasites in crop raiding and wild foraging *Papioanubis* in Nigeria. *International Journal of Primatology*. 27: 1519-1533.
- Wolf D, Vrhovec M, Failing K, Rossier C, Hermosilla C and Pantchev N (2014) Diagnosis of gastrointestinal parasites in reptiles: comparison of two coprological methods. *Acta Veterinaria Scandinavica* 56 (44) 1-13.

VIRTUALLY DISTRIBUTING PUBLIC RELATIONS: A STUDY ON HOW VIRTUAL MEDIA REINVENT THE TRENDS OF MODERN PUBLIC RELATIONS

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Abstract

Public Relations (PR) is a strategic and deliberate communication process that builds mutually valuable relationship between organizations and the public. Public Relations comprises an organization or individual gaining exposure to their audiences using topics of public interest and news items free of cost. The aim of public relations is to inform both internal and external public like prospective customers, investors, partners, employees and other stakeholders to maintain a positive perception about the organization, its leaderships, products or political decisions.

Creation and maintenance of a good public reputation is a result of complex and ongoing Public Relations process. Without an effective PR, it is very difficult to reach the attention of the target audience and to influence their opinions and decisions. In order to make a good relationship with the target audience and keep it on a high level, PR uses a variety of tools and techniques. New communication technology has replaced traditional PR tools with online tools like social networks and modern apps. An extended version of traditional PR practices is visible in the modern public relations.

This paper studies the changes that have occurred in the PR industry after the emergence of new media and social networks. The notion of social networking has changed the way PR worked. Traditional ways of PR practice has gone obsolete and new trends hit the industry with new possibilities. This study throws light in to the new virtual PR scenario and it analyses the new trends emerged as part of the proliferation of digital PR in our country.

Key Words: Public Relations, PR, Digital Media, Virtual PR, Modern PR industry, New trends in PR, Social Media

Public Relations has come a long way since its birth. This two-way communication which establishes the mutual understanding between the organisation and its public has undergone tremendous changes over the past decade alone. Public Relations students still study the ethics, code of conduct and traditional methods of PR practices in their curriculum. But the industry is more focusing on the utilization of technological advancements in the field of communication. Public Relations manages the spread of the information between an individual or an organization such as a business, government or a non-profit organization and the public.

New media have had a huge impact on the practice of public relations since the first web blog, or blogs appeared years ago. Since then, the communications media have developed into different forms including text, images, audio and video through the increase in forums, message boards, photo sharing. New media like computers, internet, virtual world, computer animation and interactive computer installations have also changed the face of PR.

Social sites, a recent phenomenon in the new media have also changed the way people communicate, as well as when they communicate, where they communicate and even who they communicate with. Social media forced many brands to start focusing more on their customers, and building positive relationship. Customers can always go on to different social platforms like Twitter to Instagram to voice about bad customer experiences. This forces companies to resolve issues swiftly to avoid a crisis.

In the past, communication was a one-way affair, where companies place content and customers had no public avenue to easily interact with or respond to distributed content. Now customers engage with brands and their content by leaving comments, 'liking', 'mentioning' and 'sharing'. This also helps brands

to know how effective the content and ads are in reaching the masses, and boosting visibility, as opposed to the past where the effect of commercials and newspaper ads could only be measured in sales. Social media continue to have a strong impact on public relations, and may continue to influence the ever-changing Public Relations field.

Social media is constantly evolving, and the accessibility of live streaming options like Facebook Live, Instagram Live, YouTube Live, and many more means that brands can connect with their audiences in real time. The line between public relations, marketing and advertising is blurring. Today's PR professionals have become brand ambassadors, social media experts, content marketers, and trend spotters. Now many PR firms rebranded to integrated agencies, offering services beyond traditional PR.

Today people are quite fascinated about social media. The extensive use of social media significantly changed the way people communicate and share information. Social media have had a huge impact not only on people, but also on brands across industries as they set up strategies to engage their audiences and win their loyalty. Public Relations is of no difference as professionals constantly seek to communicate with and hear from customers, who are ever present and active on social media.

Traditionally, consumers used the Internet to simply expend content: they read it, they watched it, and they used it to buy products and services. Increasingly, however, consumers are utilizing platforms—such as content sharing sites, blogs, social networking, and wikis—to create, modify, share, and discuss Internet content. This represents the social media phenomenon, which can now significantly impact a firm's reputation, sales, and even survival.

Modern day Public Relations has evolved from what traditional Public Relations used to be. It has come a long way from banners, posters, radio, television, and marketing adverts to a 24X7 presence. Adoption of new trends is need for the survival of any PR person or company today. Now PR is everywhere. Knowingly or unknowingly we do PR for someone. People who are up to date with the industry and trends in media can do well in PR. Securing media coverage and be in the lime light of social media has become an easy task for the PR Professionals. Ethics seems to lose its integrity in the Public Relations front.

Public Relations is about stories. To tell desirable narratives to the target audience, vital data has to be gathered and scrutinized. This is mainly because of the internet age that has ensured there is so much information available on your finger tips, so many businesses starting as fast as it takes to create a logo and website, and therefore so many companies pushing for their own stories.

Previously, pitching was an easy job and mass emailing worked so effectively for it because journalists, writers, and bloggers all received very few emails and pitches. It is not the same anymore. Pitching is only effective if you take your time to study everyone in your press list, their readership and specializations. It has become more personalized and one cannot get away with mass emailing anymore because journalists and bloggers now receive a ton emails every day. In modern public relations, pitching has to be done intensely and backed by research. The startups that are focusing on pitching based on collected data get media coverage easily than those who are sending emails trying to pitch their new launch or product feature. The new wave of pitching is now personalized pitches.

Social media and its influence have become an integral part of PR, and savvy professionals use tools such as *BuzzSumo* and *Hootsuite* to use online platforms. Beyond them lurk communication methods such as text, email and chat apps, which are nearly impossible to monitor. Organizations must create, own and manage their own digital property. By developing a proprietary platform, you can build a library of unique and valuable content. This will heighten your audience's engagement, keep them coming back for more and give you visible results.

PR Trends evolved with the emergence of virtual media

Public Relations is all about managing communication between an organization its public with a focus on building and maintaining a reputation. Typically, it involves evaluating public exposure, opinions, and attitudes, implementing communication programmes across media channels and creating goodwill by managing a two-way communication process. The emergence of virtual media has influenced the traditional practices of PR and it gave way to new trends in the industry.

Influencer Marketing

The PR industry will soon face the decline in traditional media's reach and an outburst of influencer marketing. Social media influencers and influencer content will decide the success of a brand. Basically the goal of PR is to say good things about your brand. Influencer marketing is building relationship with key individuals who can reach your target audience. A relationship with the influencer can help boosting your social networking and online PR campaigns. Influencer marketing has a human element that PR lacks and it encourages potential audience to trust your brand hence it can impact your PR efforts in a great way. There are many apps which help one to structure

influencer marketing strategy. Follower Wonk helps to find the ones with biggest reach and social authority. BuzzSumo is another search influencer tool that also analyzes content shared by influencers. Mention, Twitter lists, Hey Press adds new meaning to the novel idea of Influencer Marketing. The great thing about working with influencers is that their involvement makes your brand more appealing to journalists and publications.

New digital channels imply that brands themselves can become influencers within their industry and target audience. An entrepreneur can turn into an idea head by building up an extraordinary blog, or a brand can have a viral social media profile, it just takes a little work. On the other hand, brands can work legitimately with influencers on a one-on-one premise to develop unique exclusives that will resonate with their target audience. Brands can either connect directly with audiences through their own branded channels or through influencers with an already-established reach. Building trust through direct relationships is important when it comes to establishing a brand.

New advanced channels imply that brands themselves can move toward becoming influencers inside their industry and target group of spectators. A viral internet based life profile will just take a little work. On the other hand, brands can work legitimately with influencers on a one-on-one premise to create one of a kind special feature that will resound with their intended interest group. There is a progressively included innovative procedure when assembling an effective influencer system. Brands can either interface straightforwardly with spectators through their very own marked channels or through influencers with an effectively settled reach. Building trust through direct connections is significant with regards to setting up a brand.

Partner Role

PR can move to a position of a strategic partner instead of pitching press releases. PR will become a content source for the media for news stories.

Broader Role

PR professionals will move beyond once and done activities like press releases, press conferences and media pitching. PR professionals will develop on-going relationships with their target audiences across interest segments. As a result, they'll expand and deepen their reach by creating share of audience attention using consistent content distributed across multiple channels. To make it more effective, PR professionals will use the power of storytelling sourced from employees across the organization. Adding this human element will result in improved trust in future. Lastly, PR professionals can develop information rich message by converging visual, video and voice content based on the context.

Artificial Intelligence and PR

Artificial Intelligence is a collection of tools and technologies to make machines smart. When it comes to PR it can do a lot. It helps in tackling crisis and foreseeing media trends. Today, PR companies have access tonnes of consumer data and they fail to process this data according the consumer pattern. With insights on consumer behavior, companies can deliver more targeted messages to their clients. The world is riding on customization and brands are adopting occupational advancements to provide tailor-made products and services.

There has been a slow but stable approach towards the use of AI by PR professionals. PR professionals can make use of many benefits of AI. One of

the important areas where AI can help is PR campaigns. Data-Driven PR Campaigns uses Artificial Intelligence to explore through an immense amount of data, and find out the features that can eventually lead to a successful campaign. With AI's machine learning and automation, PR professionals don't need to do guesswork anymore. Instead, their decisions are made on facts and insights. AI also allows PR professionals to make data-driven decisions like evaluating the best time to start a campaign, where to deploy it, which bloggers to engage and so on.

Storytelling and Relationship Building

"The two most important skills for a PR professional are storytelling and relationship building which haven't changed. But the ways those skills are being deployed is changing. Beyond the media and even industry influencers, PR professionals will expand the audiences they work with to include a broader set of partners: vertical websites, content syndicators, complementary product vendors, trade associations, and others who can help bring their stories to a wider audience."

Major PR trends for the next five years are digital storytelling, social monitoring and big data. To align your PR and marketing with this changing world companies need to use digital storytelling to engage the audience. This will facilitate outsiders get to know the people within the company, and inform them about what sets you apart from you competitors. Share your passions and your struggles. This kind of narrative will endear you to prospects and keep them engaged in your story. Become an ace at social monitoring with apps like *Be the first* to see negative conversations about your brand, and be ready to address problems before they get out of hand.

Earned Media Expand and PR Measurement Improves

Earned media means more than press releases and traditional media news stories. The meaning of earned media has changed and expanded with the rise of online influencers and social media. It also includes social media mentions, mentions in blog posts, web searches, review sites, word of mouth. It is any positive mention through a channel the brand doesn't pay for or own.

PESO Media Model

Communication professionals are increasingly turning to the PESO model when planning their PR campaigns. The PESO model, developed by Gini Dietrich, lead blogger at the PR and marketing blog Spin Sucks, identifies the four media types — Paid, Earned, Shared, and Owned and merges them together. PR people are the best content creators and storytellers and have the unique opportunity to embrace the entire PESO model for clients. Moving to such an inbound world is inevitable in our digital reality."

PR will continue to be a trusted partner to media. It's no secret that the media landscape has changed dramatically over the past decade. Gone are the golden days of print and instead, journalists are under increasing pressure to produce results and get digital content out there at a rapid rate. Public relations professionals will therefore continue to act as valuable content partners for the media, providing assets they can use to develop meaningful stories.

References

Balan, K. R. (2015). *Principles Of Public Relations*. New Delhi: Himalaya Publication House.

Lister, M. (2010). *New media: A critical introduction*. London: Routledge.

- Morgan, C. (2013, April 22). What is Digital PR? Retrieved from <https://www.socialmediatoday.com/content/what-digital-pr>
- Rise, A., & Rise, L. (2004). *The Fall of Advertising and the Rise of PR*. New York, United States: HarperCollins.
- Social media. (2019, June 1). Retrieved from https://en.wikipedia.org/wiki/Social_media
- Swallow, E. (2010, August 16). The Future of Public Relations and Social Media. Retrieved from <https://mashable.com/2010/08/16/pr-social-media-future/>
- Wilcox, D. L., Cameron, G. T., & Reber, B. H. (2016). *Public relations: Strategies and tactics*. Boston: Pearson.

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Bull AT (2004) *Microbial diversity and bio prospecting*. ASM press, New York

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