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Effect of an External Magnetic field on regeneration in *Hydra vulgaris Ind Pune*

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Abstract
Exposure to magnetic fields has been reported to have beneficial as well as harmful effects. Studies indicate biomagnetism to be a helpful regeneration therapy for chronic health conditions whereas some studies suggest links between exposure to electromagnetic fields and various harmful effects (Kho *et al.*, 2008; Khaya MC *et al.*, 2014; Gerardi *et al.*, 2008; Kim *et al.*, 2013) [7, 8, 4, 3]. This study aimed at determining whether there is an effect of magnetic field exposure on regeneration time in *Hydra vulgaris*, which is a simple multicellular model system. The animals were bisected, exposed to 6 mili Tesla and 12 mili Tesla intensities of 50 Hz magnetic field for 30 seconds and 60 seconds each and the length of the regenerated head and foot was measured over two days using the ImageJ software and compared with the control. Statistical t-test analysis of the data revealed no difference between regeneration in the control and exposed *Hydra vulgaris Ind Pune.*

Keywords: *Hydra vulgaris Ind Pune*, Biomagnetism, Regeneration, Electromagnetic field.

1. Introduction
Biomagnetism has been shown to help the body heal itself of even chronic and long-term conditions. Applied magnetic fields have been shown to enhance nerve regeneration in mammals and other organisms. Recently, studies have revealed that magnetic field exposure could inhibit cell growth of prostate cancer cells (Kho *et al.*, 2008) [7], induce cell apoptosis of breast cancer cells (Kahya MC *et al.*, 2014) [8], and affect reproduction of animals (Naziroglu *et al.*, 2013) [9]. Applied electromagnetic fields of 50 Hz and 0.5 mT was also shown to decreases oxidative stress induced by global cerebral ischemia and reduced possible negative consequences of free radical species in the brain. (Balind *et al.*, 2014) [1].
Along with the beneficial effects, magnetic field exposure is known to have harmful effects.
Magnetic fields are produced by electrical appliances, power lines, electromagnets and everything that carries electrical current. The use of devices generating high magnetic fields in industries, medical diagnostics, vehicles and research industries has considerably increased and is expected to increase further in the near future. Several epidemiological studies suggest links between exposure to electromagnetic fields and various harmful effects. These fields can affect a wide range of biological processes, including growth and development.
The widespread exposure to electromagnetic fields in our environment and its biological effects has attracted the attention of researchers. Extremely low frequency magnetic field exposure has been found to have detrimental effects on the embryonic development of zebra fish by affecting hatching, decreasing the heart rate, and inducing apoptosis but these effects were a not mortal threat to the fishes (Ying Li *et al.*, 2014) [2]. Long term exposure to electromagnetic fields with a well-defined frequency had relevant effects on parameters such as body weight, blood glucose and fatty acid metabolism in Rats (Gerardi *et al.*, 2008) [4]. One study suggested no effects on heart rate, respiration rate, and heart rate variability observed in adults or teenagers resulting from 32 min of 60 Hz and 12.5 μT magnetic field exposure (Kim *et al.*, 2013) [3].
The purpose of this study was to check whether *Hydra* after bisection would show any changes in regeneration profile of the head and foot segments with respect to the length of the regenerated tissue when exposed to different intensities of 50 Hz magnetic fields for specific time intervals.

2. Materials & Method

I] Procurement of Hydra Vulgaris Ind Pune

The *Hydra* culture was obtained from Dr. S Ghaskadbi's lab at Agharkar Research Institute in Pune.

II] Culturing *Hydra Vulgaris Ind Pune*

Hydra Vulgaris Ind Pune cultures were maintained in 100ml beakers containing Hydra Medium (HM) prepared according to Sugiyama and Fujisawa at a constant temperature of 18ºC with a 12 hour light/dark cycle (Sugiyama *et al*., 1977) [6]. The hydra polyps were fed with freshly hatched *Artemia nauplii* every 2 days followed by changing the media and replenishing the *Hydra* with fresh media 4-6 hrs post feeding.

III) Exposure to Magnetic Field

Individual *Hydra* polyps were placed on a clean glass slide and observed under 4X magnification in dark field filter of the Olympus microscope. *Hydra* polyps were allowed to extend and photographs of the intact *Hydra* were obtained. *Hydra* polyps were cut approximately at the centre using a sharp blade. Photographs were obtained post cutting after the *Hydra* fragments relaxed. The experimental set of *Hydra* fragments were placed in a specially designed glass trough (Appendix 1) and then exposed to 6mT and 12mT magnetic field intensities for 30seconds and 60seconds using a DC Power Pack of 5 Volt. Different values of magnetic field at different places in the trough were estimated using a standard Gauss meter and then converted to mili Tesla (Appendix 2) Control *Hydra* fragments were not exposed to magnetic fields. In set I, 5 Hydra were used for each magnetic field intensity and 5 Hydra were used as Controls. In set II, 6 Hydra were used for each magnetic field intensity and 20 Hydra were used as Controls. *Hydra* head and foot segments were placed separately and numbered in sterile 6 well culture plates (*Nunc* make) and the regeneration of these fragments was monitored for 2 consecutive days. The length of the *Hydra* in each photograph was measured using the ImageJ software in pixels and converted to mm (1mm=548.45pixels).

3. Results

Table 1: Mean±sd and t-test for Set I & Set II Heads (Set I Control: N= 5, Set I Exposed: N=5; Set II Control: N=20, Set II Exposed: N=6)

	Set I Heads				**Set II Heads**			
	Mean Difference in lengths (mm) ±sd	**T calc**	**T stat$_{(0.05,8)}$**	**Inference**	**Mean Difference in lengths (mm) ±sd**	**T calc**	**T stat$_{(0.05,24)}$**	**Inference**
Control	0.2359±0.229				0.0705±0.073			
6mT 30s	0.0604±0.154	0.1990	2.306	No Difference	0.6955±0.509	0.0299	2.064	No Difference
6mT 60s	0.0628±0.065	0.1699	2.306	No Difference	0.0571±0.085	0.7400	2.064	No Difference
12mT 30s	0.7669±0.492	0.0744	2.306	No Difference	0.0201±0.083	0.2226	2.064	No Difference
12mT 60s	-0.0224±0.144	0.0721	2.306	No Difference	0.0756±0.068	0.8766	2.064	No Difference

Table 2: Mean±sd and t-test for Set I & Set II Foot (Set I Control: N= 5, Set I Exposed: N=5; Set II Control: N=20, Set II Exposed: N=6)

	Set I Foot				**Set II Foot**			
	Mean Difference in lengths (mm) ±sd	**T calc**	**T stat$_{(0.05,4/5/6)}$**	**Inference**	**Mean Difference in lengths (mm) ±sd**	**T calc**	**T stat$_{(0.05,24)}$**	**Inference**
Control	0.4889±0.178				0.0563±0.130			
6mT 30s	0.3301±0.071	0.2595	2.776	No Difference	0.6276±0.516	0.0561	2.064	No Difference
6mT 60s	-0.0441±0.087	0.0217	2.571	No Difference	0.0429±0.156	0.8533	2.064	No Difference
12mT 30s	0.5145±0.124	0.8402	2.447	No Difference	0.1043±0.101	0.3656	2.064	No Difference
12mT 60s	0.0069±0.155	0.0198	2.571	No Difference	0.0886±0.102	0.5423	2.064	No Difference

Table 3: T-test for comparison between Set I and Set II Heads & Set I and Set II Foot at different magnetic field intensities and same time intervals & same magnetic field intensities and different time intervals.

	HEADS				**FOOT**			
Comparison between		T calc	T stat$_{(0.05,8/10)}$	INFERENCE		T calc	T stat$_{(0.05,6/10)}$	INFERENCE
6mT 30s and 12mT 30s	SET I	0.0298	2.306	No Difference	SET I	0.0373	2.447	No Difference
	SET II	0.0222	2.228	No Difference	SET II	0.0729	2.228	No Difference
6mT 60s and 12mT 60s	SET I	0.2782	2.306	No Difference	SET I	0.5930	2.447	No Difference
	SET II	0.6888	2.228	No Difference	SET II	0.5659	2.228	No Difference
6mT 30s and 6mT 60s	SET I	0.9761	2.306	No Difference	SET I	0.0017	2.447	No Difference
	SET II	0.0273	2.228	No Difference	SET II	0.0522	2.228	No Difference
12mT 30s and 12mT 60s	SET I	0.0205	2.306	No Difference	SET I	0.0021	2.447	No Difference
	SET II	0.2377	2.228	No Difference	SET II	0.7952	2.228	No Difference

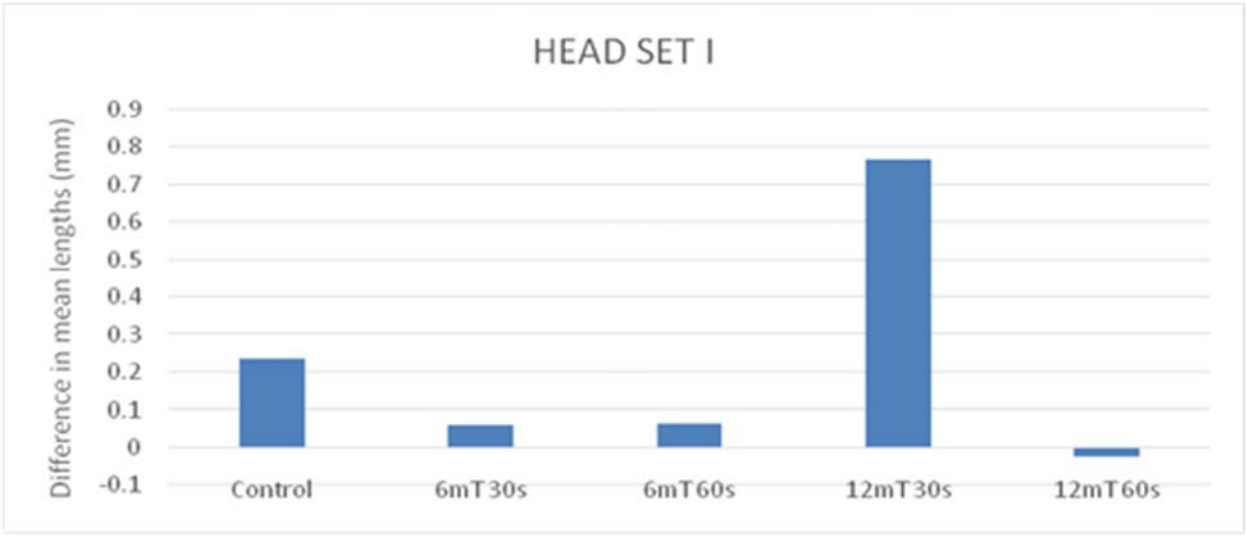


Fig 1: Graphical representations for difference in mean lengths between Day 2 & Day 0 in Control and Different magnetic field intensities in SET I Heads.

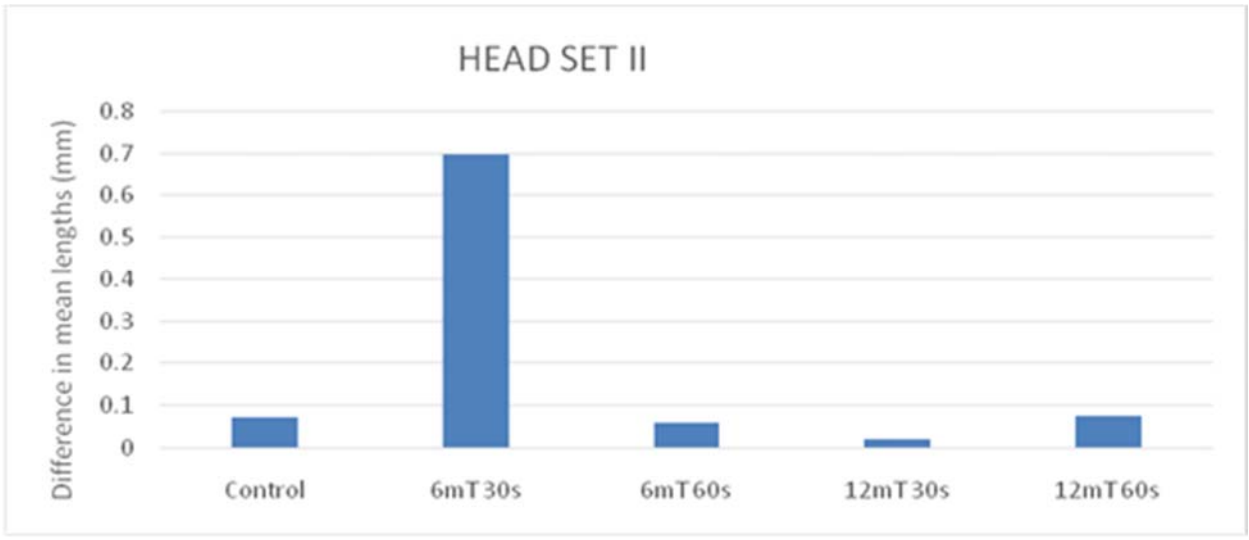


Fig 2: Graphical representations for difference in mean lengths between Day 2 & Day 0 in Control and Different magnetic field intensities in SET II Heads.

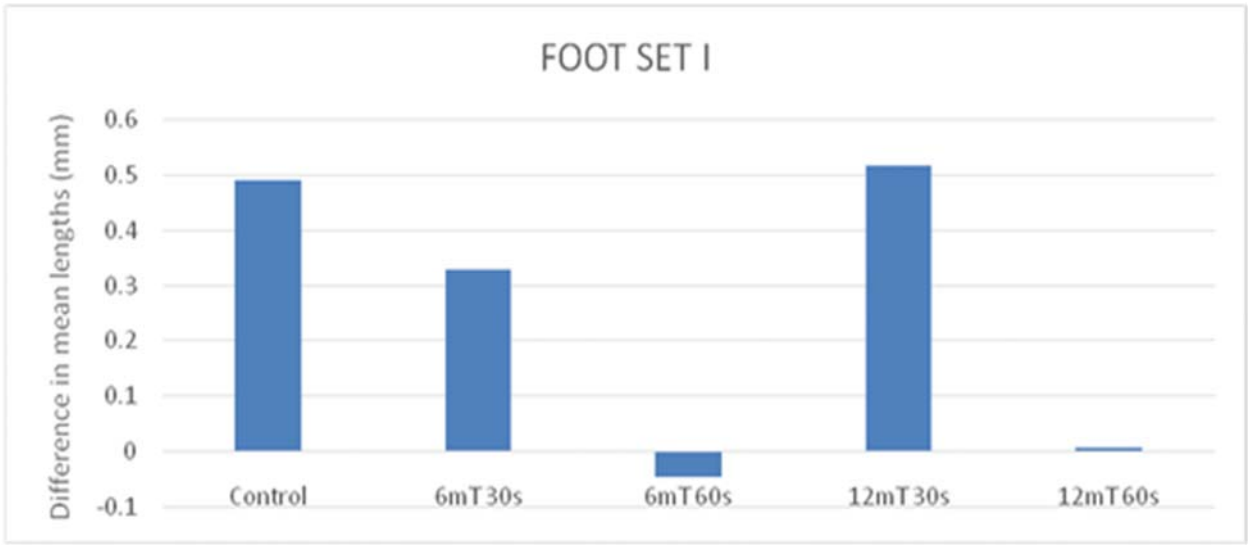


Fig 3: Graphical representations for difference in mean lengths between Day 2 & Day 0 in Control and Different magnetic field intensities in SET I Foot.

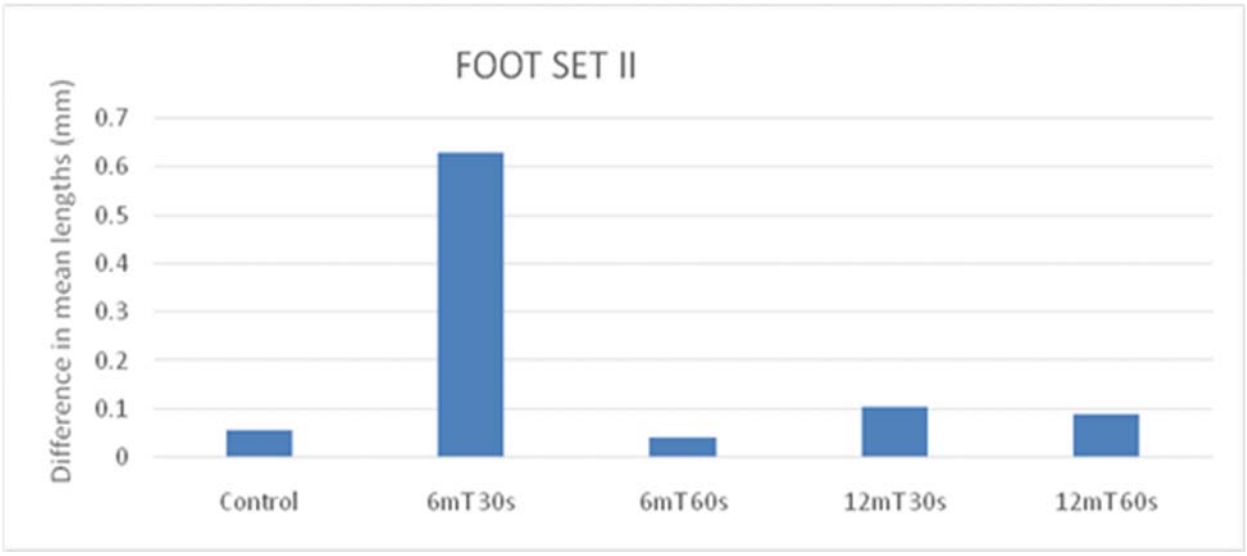
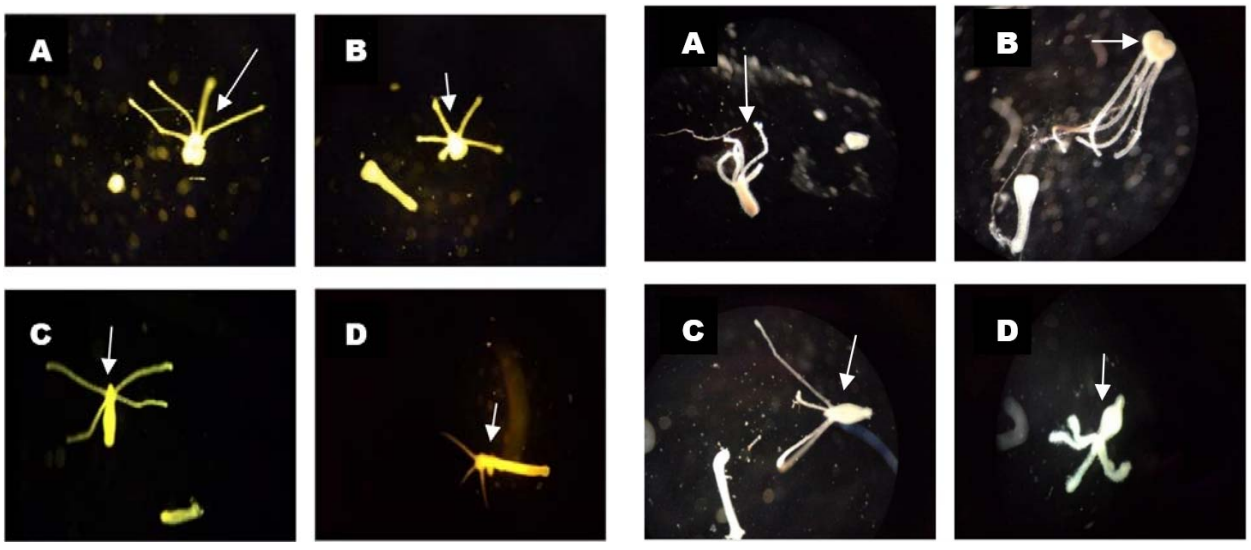


Fig 4: Graphical representations for difference in mean lengths between Day 2 & Day 0 in Control and Different magnetic field intensities in SET II Foot.



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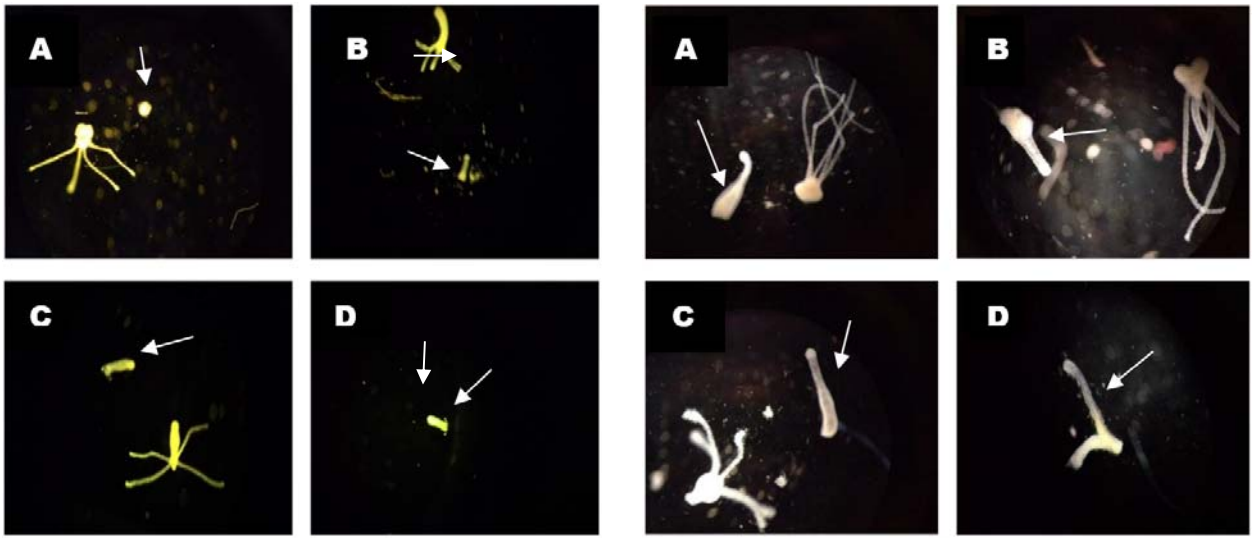

III IV

Fig 5: (548.45pixels=1mm)**:**

I - Set I Heads (IA – Day 0 Control, IB - Day 0, 12 mT 30 seconds exposure, IC - Day 2, Control, ID - Day 2, 12 mT 30 seconds exposure.) II - Set II Heads (IIA - Day 0, Control, IIB - Day 0, 6mt 30 seconds exposure, IIC – Day 2, Control, IID - Day 2, 6mt 30 seconds exposure.) III - Set I Foot (IIIA - Day 0 Control, IIIB - Day 0, 12mt 60 seconds exposure, IIIC - Day 2, Control, IIID - Day 2, 12mt 60 seconds exposure.) IV - Set II Foot (IVA - Day 0 Control, IVB - Day 0, 6mt 30 seconds exposure, IVC - Day 2, Control, IVD - Day 2, 6mt 30 seconds exposure.) [Arrows point to the regenerating head/foot segments taken into consideration]

On analyzing the mean difference in lengths of the *Hydra* between Day 2 and Day 0, it can be observed that regeneration of the cut heads in Set I was the highest at 12mT 30 seconds magnetic field exposure with respect to the Control (Refer to Fig 1 & 5-I). In Set II, the regeneration of the cut heads was the highest in 6mT 30 seconds magnetic field exposure with respect to the control (Refer to Fig 2 & 5-II). Analysis of the regenerating foot segments in Set I, exhibited no difference in the mean lengths between Day 2 and Day 0 of the Control and 12mT 30 seconds magnetic field exposure (Refer to Fig 3). However 12mT 60 seconds magnetic field exposure resulted in a decrease in the regenerating lengths compared to the Control (Refer to Fig 3 & 5-III). In the regenerating foot segments of Set II, the difference in the mean lengths between Day 2 and Day 0 was the highest for 6mT 30 seconds exposure (Refer to Fig 4 & 5-IV). This discrepancy in the results may have occurred since the Set I *Hydra* used for the experiment exhibited early signs of degeneration.

Statistical t-test analysis was conducted on the data obtained and the results showed no significant difference between the regeneration of the *Hydra* in the Control samples and the 6mT 30seconds, 6mT 60 seconds, 12mT 30 seconds, 12mT 60 seconds exposed samples for both the head and foot segments (Refer to Table 1 & 2). Statistical t-test analysis for comparison between regeneration in Set I and Set II Head and Foot at different magnetic fields and same time exposure as well as same magnetic field and different time exposure revealed no significant difference (Refer to Table 3).

The experiments thus suggest that there were no statistically significant differences seen in the lengths of regenerating head and foot segments of *Hydra* over a period of 24 & 48 hours, when compared with the Controls.

4. Discussion

Our study aimed at determining whether exposure to different magnetic field intensities at specific time intervals affect the rate of regeneration in the head and foot segments of a bisected *Hydra*. Statistical t-test analysis of the data obtained indicated no significant difference in the regeneration between the control *Hydra* and *Hydra* exposed to magnetic fields. In a study on Urban Ecosystem as a superposition of interrelated active media, a negative effect on regeneration was observed in *Hydra oligactis* when subjected to 50Hz magnetic field intensities between 580-1960A/m (A.E Sidorova *et al.*, 2014) [12]. In another study, extremely low frequency magnetic field exposure was found to have detrimental effects on embryo development in Zebra Fish (Ying Li *et al.*, 2014) [2]. Long term exposure to electromagnetic fields with a well-defined frequency had relevant effects on parameters such as body weight, blood glucose and fatty acid metabolism in Rats (Gerardi *et al.*, 2008) [4]. Hydra Metalloproteinases 1 and 2 were found to be important in head and foot regeneration respectively (Yan L *et al.*, 2000; Yan L *et al.*, 1999) [10, 11]. The effect of extremely low electromagnetic fields was also reported to decrease metalloprotein redox active sites (Ninno *et al.*, 2008) [5]. Therefore magnetic fields were proposed to have some effect on regeneration in *Hydra*.

5. Conclusion

Our study thus concludes with the observation that regeneration of the head and foot segments in a bisected Hydra shows no statistically significant difference when compared to the Control on exposure to 6mT and 12mT magnetic field intensities for 30seconds & 60seconds.

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7. References

1. Balind S, Selakovic V, Radenovic L, Prolic Z, Janac B. Extremely low frequency magnetic fields (50HZ, 0.5mt) reduces oxidative stress in the brain of Gerbils submitted to Global Cerebral Ischemia. PLOS ONE. 2014; 9(2):1-10.
2. Li Y, Lui X, Lui K, Maio W, Zhou C, Li Y *et al*. Extremely low frequency magnetic fields induce developmental toxicity and apoptosis in Zebra fish embryos. Biol Trace Elem Res 2014; 162:324-332.
3. Kim S, Choi J, Kwon M, Choi J, Kim D. Effects of 60 Hz magnetic fields on teenagers and adults. Environmental Health 2013; 12:24.
4. Gerardi G, De Ninno A, Prosdocimi M, Ferrari V, Barbaro F, Mazzariol S *et al.* Effects of electromagnetic fields of low frequency and low intensity on rat metabolism. Bio Magnetic Research and Technology 2008; 6(3):1-8.
5. Effect of ELF e.m fields on metallo protein redox-active sites. http://arxiv.org/ftp/arxiv/papers/0801/0801.2920.pdf., 2008.
6. Sugiyama T, Fujisawa T. Genetic analysis of developmental mechanisms in Hydra III Characterization of a regeneration deficient strain. J Embryol Exp Morphol. 1977; 42:65-77.
7. Koh EK, Ryu BK, Jeong DY, Bang IS, Nam MH, Chae KS. A 60-Hz sinusoidal magnetic field induces apoptosis of prostate cancer cells through reactive oxygen species. Int J Radiat Biol. 2008; 84(11):945-955.
8. Kahya MC, Nazıroğlu M, Çiğ B. Selenium reduces mobile phone (900 MHz)-induced oxidative stress, mitochondrial function, and apoptosis in breast cancer cells. Biol Trace Elem Res 2014; 160(2):285-293.
9. Nazıroğlu M, Yüksel M, Köse SA, Özkaya MO. Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signalling pathways in females and males. J Membr Biol. 2013; 246(12):869-875.
10. Yan L, Lenotovich A, Fei K, Sarras M. Hydra

Metalloproteinase 1: A secreted astacin metalloproteinase whose apical axis expression is differentially regulated during head regeneration. Developmental Biology 2000; 219:115-128.
11. Yan L, Fei K, Zhang J, Dexter S, Sarras M. Identification and characterization of HMP-2: a meprin-like astacin metalloproteinase that functions in foot morphogenesis. Development 2000; 127:129-141.
12. Sidorova AE, Mukhartova YU, Yakovenko LV. An Urban Ecosystem as a Superposition of Interrelated Active Media. Moscow University Physics Bulletin 2014; 69(5):392-400.