



A STUDY ON THE EFFECTS OF CLEANING AGENTS (HOUSEHOLD) ON SEED GERMINATION

S. Vanitha

Assistant Professor, Department of Biochemistry
Bhavan's Vivekananda College of Science, Humanities and
Commerce
Hyderabad, Telangana, India.

L. Vighnesh

Department of Biochemistry
Bhavan's Vivekananda College of Science, Humanities and
Commerce
Hyderabad, Telangana, India.

V. Sreekar

Department of Biochemistry
Bhavan's Vivekananda College of Science,
Humanities and Commerce Hyderabad, Telangana, India

Abstract: Germination of seeds is dependent on fertility of the soil but, from 1940 onwards and till now household cleaning agents are used commonly. They contain a surfactant which chemically reacts with greasy substances and also contain harsh chemicals with perfume and colour. These potentially hazardous substances when used frequently or disposed of by pouring them through drains or even dumping them directly into the ecosystem could seep into the water supply that plants depend on and affect their normal development and growth. The aim of this study was to determine the effects of various cleaning agents on germination of seeds and a proposal for the design of biosensors to monitor the fertility of the soil or water directly and indirectly. Seeds were germinated in a petri plate containing various concentrations of detergents, cleaning agents and laundry blue. The results were cleaning agents at higher concentration has reduced seedling growth and also its seed vigour index were less when compared to the control. No germination was observed in presence of laundry blue, floor disinfectant showed low germination percentage and least growth was observed in stiffener and soap solution. The developed seeds showed difference in colouration and their root and shoot lengths were short and coiled. A biosensor can be designed using photosystem II which can bind with potential chemicals from household effluents present in the soil or water which is used for irrigation. As an indirect method a nano biosensor can be used to quantitatively measure the differential oxygen consumption in the respiration of "good microbes" and "bad microbes" in the soil and their load, pH, humidity and soil constituents.

Keywords: Biosensors, germination, photosystem II, seed vigour index, surfactant.

I. INTRODUCTION

Agriculture plays a vital role in India's economy. Over 58% of the rural households depend on agriculture as their principal means of livelihood. Green revolution was characterized by use of high yielding variety of seeds and fertilizers increased productivity but, there is a state of decline in productivity which is due to changes in environment in the form of depleting water table, emission of greenhouse gases and the contamination of surface and ground water damaging the soil fertility. Availability and accessibility of wet land and water resources are decreasing creating a decline in agricultural output. Water companies, food manufacturers and farmers are experiencing pressure from consumers with increased demands in the supply of potable water, high quality in food free from pollutants and food with accurate nutritional values. A major threat to human health is increase of pollutants in soil, water resources and food coming from regular use of different cleaning agents. The situation now demands development and design of rapid, cost effective, miniature sensing devices to monitor the levels of chemicals in the soil to promote sustainable agriculture with reduction in farm cost and increase product values [1]. Soil sample analysis is regularly done as 'off-site' by sending to laboratory for testing, which has higher accuracy but with low detection limits, expensive, time consuming and require the use of a trained personnel. But the need to analyze the samples 'on site' has increased the demand with simple, robust, rapid and low cost technologies

where the conventional analytic methods can be replaced with biosensors. This modern microelectronics can increase the detection limits, accuracy in results, many parameters analyzed in single step, speed and the equipment must be handy [2]. In the field of medical diagnostics biosensors are used successfully but in the field of food, agriculture, veterinary diagnosis and environmental assessments are still to be established which is a big challenge. A biosensor principle is detection of compounds where an analyte binds specifically to its complementary bio recognition element immobilized on a support medium. This interaction causes few changes in the physical and chemical properties which are detected, amplified and measured by a transducer. The electronic signal developed is dependent on the concentration of the analyte in the sample [3], [4]. Biosensors are applied in various disciplines like medicine, industry, environmental analysis, food technology and military [5].

The early development of germinating seeds is dependent on uptake of water, fertility of the soil and various other favorable environmental factors. But from 1940 onwards, till now and may be in future also household cleaning agents are going to be used regularly and commonly. These cleaning agents include soap bars and liquid gels used for washing hands, face or body, detergent powders or cakes for cleaning laundry items, household cleansers are used to clean furniture, glass, plastic items and to clean kitchen dishes and utensils dish cleaning products are used daily [6]. The common ingredients in all these cleaning agents are fat, alkali, glycerin, surfactants or surface active agents, and also

detergent boosters, builders, fillers, film removers, dry cleaning fluid, bleaches, ammonia all added together with perfume and color. They mainly remove greasy substances and dirt. Few potentially harmful substances containing agents when used frequently or disposed of by pouring them through drains or even dumping them directly into the ecosystem could seep into the water supply that plants depend on and affect their normal development and growth.

Pollutants from fertilized fields, municipal sewage and industrial effluents contain phosphates and nitrates [7]. During favorable environmental conditions these compounds encourage the growth of microorganisms resulting in eutrophication [8], and result in different odor, deteriorates taste of water, growth of algae that may be harmful, and overall it affects the filtration of water and increases the cost of water treatment [9], [10]. Photosynthesis in plants is an important event for its normal development, but exposure to detergents can have a huge effect causing extensive changes either in their root or shoot length and secondary roots development. Detergents can induce denaturation of the reaction centers of photosystems[11], drastically change the fluorescence properties of light harvesting complexes of photosystem II [12], at low concentration they can saturate the photosynthetic reactions centers [13] and relaxation dynamics of photosystem II can be affected [14]. The aim of this study was to study the effect of different cleaning agents on seed germination and to propose a design of a biosensor which could detect the changes in water or in the soil.

II. MATERIALS AND METHOD

Seeds of *Vigna radiata* (Mung bean), petri plates, pipettes, 1 % sodium hypochlorite solution samples like soap and soap solution, stiffener, liquid blue, cloth freshener, hand wash, floor disinfectant and natural samples like besan powder and reetha seeds were purchased from the local market.

Seeds of *Vigna radiata* (Mung bean) were surfaced sterilized using 1% sodium hypochlorite solution for 5 minutes to prevent fungal infection and then washed three to five times with distilled water. All the commercially purchased and natural samples were diluted 1 in 10 and 1 in 100 with distilled water. Surface sterilized seeds were placed on petri plates containing two layers of filter paper wetted with 5ml of tap water (control) and 5ml of each diluted samples respectively. Ten seeds were counted and placed evenly on the plates. The plates were covered and kept for incubation at room temperature. Germination was monitored for eight days and each day no of seeds germinated was noted down. Seed vigor index (SVI) was calculated by measuring the root length (in cm) and the number of secondary roots were counted for all the germinated seeds. The results are presented as average values of the germinated seeds.

$SVI = [(Germination\ percentage \times Root\ length\ (cm)) + No\ of\ secondary\ roots]$ [15].

III. RESULTS

The experimental results are represented as follows; Table 1 and 2 shows the average measurements of

germinated seeds root, shoot length, germination percentage and SVI.

Fig 1, represents germination of seeds for eight days. In the presence of 1 in 10 diluted samples, on day 1 and 2 in all the samples few seeds germinated but from day 3 onwards in samples like herbal shampoo, hand wash, floor disinfectant, reetha, soap solution and laundry blue the seeds started to turn brown and the radicle length was 1mm only and slowly decayed.

Fig 2, Germination percentage was 100% in control, besan and cloth freshener, 90 % in distilled water, stiffener and soap whereas in herbal shampoo, hand wash, floor disinfectant, reetha, soap solution and laundry blue showed 0%.

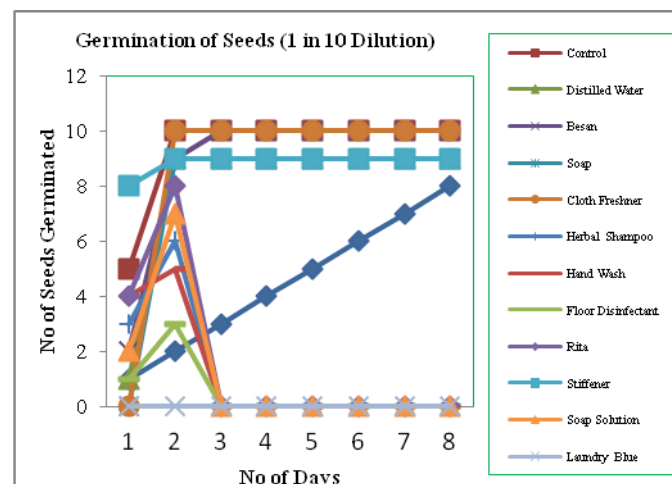


Fig 1 represents the germination of seeds in 1 in 10 dilution monitored for eight days.

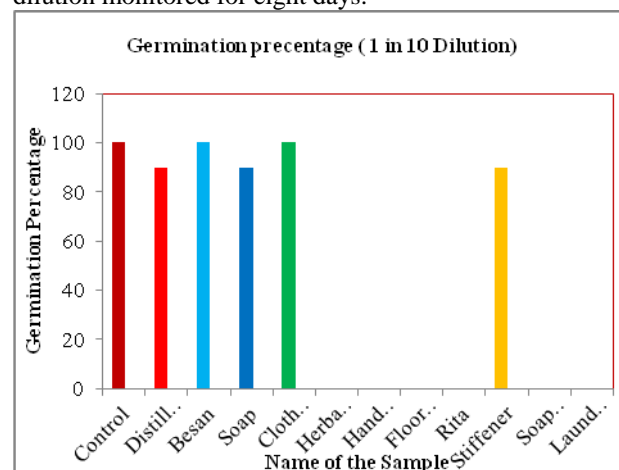


Fig 2 represents the germination percentage (1 in 10 dilution)

Fig 3. Represents Seed Vigor Index. To calculate the SVI germination percentage, root length (in cm) was measured and number of secondary roots was counted for all the germinated seeds and SVI of each seed was determined individually and the average of SVI was represented graphically. The SVI was 331.8 in control (tap water) and least 154.71 in the presence of cloth freshener. Natural

sample besan showed SVI as 342.8 slightly higher than control. SVI was not calculated for samples which exhibited zero germination percentage.

The results obtained for samples diluted 1 in 100 are as follows, Fig 4, Germination of seeds for eight days in the presence of 1 in 100 diluted samples, initially there was a delay in germination in distilled water, soap, herbal shampoo, stiffener, laundry blue and after eight days the seeds had developed root and shoot and the germination percentage was zero for laundry blue, 90 % in distilled water and soap when compared to all the other samples which showed 100 % germination Fig 5.

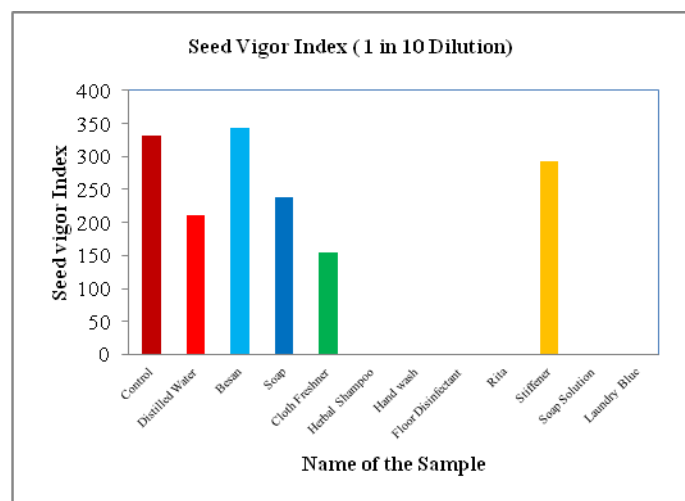


Fig 3 Seed vigor index of germinated seeds (1 in 10 dilution)

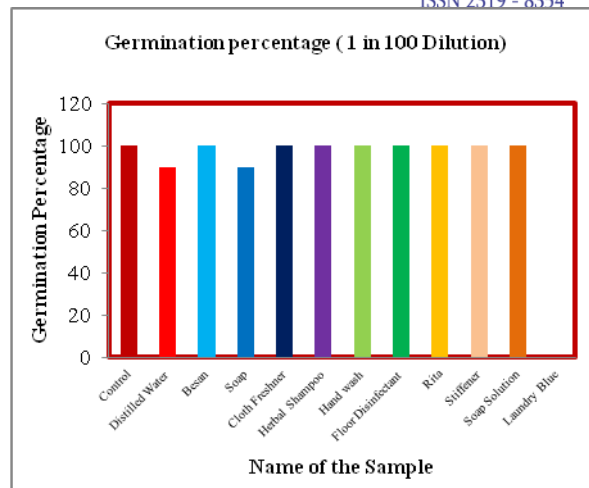


Fig 5, represents the germination percentage (1 in 100 dilution).

Fig 6, represents SVI in 1 in 100 dilution. To calculate the SVI measurements were followed as previously, the SVI was 331.8 in control (tap water), 421.3 in besan, and zero in presence of laundry blue and less SVI was found to be 210.7, 224, 232.8, and 284.2 in distilled water, hand wash, soap solution and soap respectively.



Fig 7 shows the pictures of germinated seed in tap water (Control), seeds germinated in presence of 1 in 10 diluted sample and last photo in presence of 1 in 100 dilution.

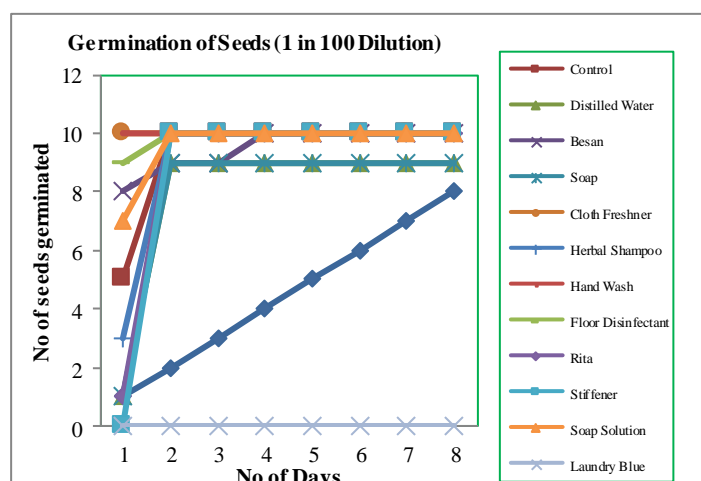


Fig 4, represents the germination of seeds in 1 in 100 dilution monitored for eight days.

Fig 6, Seed vigor index of germinated seeds (1 in 100 dilution)

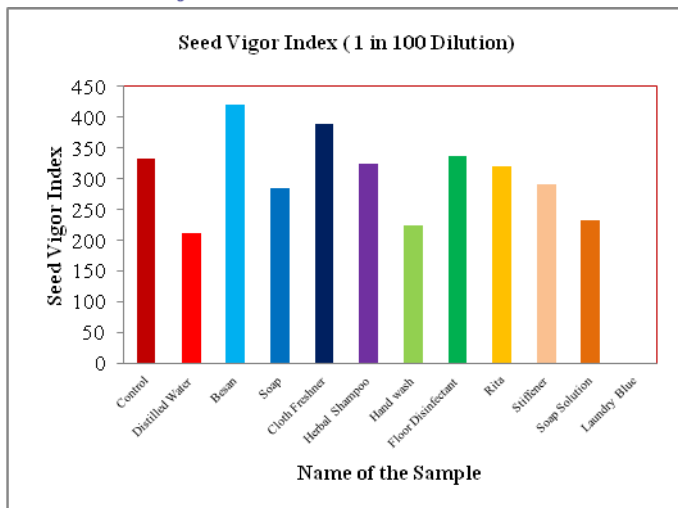


Table 1 : Represents the root and shoot length, germination percentage and SVI (1 in 10 dilution)

Sample	Root Length (in cm)	Shoot Length (in cm)	No of Secondary Roots	Germination Percentage	Seed Vigor Index (SVI)
Control	3.17	10.07	14.8	100	331.8
Distilled Water	2	2.27	10.7	90	210.7
Besan	3.3	10.6	12.8	100	342.8
Soap	2.32	3.36	5.4	90	237.4
Cloth Freshener	1.47	1.21	7.57	100	154.7
Herbal Shampoo	0	0	0	0	0
Hand wash	0	0	0	0	0
Floor Disinfectant	0	0	0	0	0
Reetha	0	0	0	0	0
Stiffener	3.1	7.18	14.2	90	293.2
Soap Solution	0	0	0	0	0
Laundry Blue	0	0	0	0	0

Table 2 : Represents the root and shoot length, germination percentage and SVI (1 in 100 dilution)

Sample	Root Length (in cm)	Shoot Length (in cm)	No of Secondary Roots	Germination percentage	Seed Vigor Index (SVI)
Control	3.17	10.07	14.8	100	331.8
Distilled Water	2	2.27	10.7	90	210.7
Besan	4.1	7.5	11.3	100	421.3
Soap	3.03	7.52	11.2	90	284.2
Cloth Freshener	3.66	10.8	24.1	100	390.1
Herbal Shampoo	3.2	7.3	5	100	325
Hand wash	2.15	5.6	9	100	224
Floor Disinfectant	3.35	6.4	20.2	100	337.2
Reetha	2.98	7.01	21.4	100	320
Stiffener	2.71	10.1	19.2	100	290.6
Soap Solution	2.2	5.42	12.8	100	232.8
Laundry Blue	0	0	0	0	0

IV. DISCUSSION



Many earlier studies have indicated the adverse effects of detergents on plant development like sodium dodecyl sulphate (SDS), house hold synthetic detergents (HDS) on diatom [16], loss of enzyme activity [17] and chlorosis in *Vigna radiata* [18]. The effect of detergent on plants varies depending on how the plant is exposed to it where growth was inhibited followed by loss of metabolic activity within 5 days [19], modification in the membrane biophysical properties [20], cell growth inhibition and blocking chlorophyll synthesis in presence of household synthetic abstergent [21]. Presence of sodium sulphate, nitrogen and its oxide and phosphates in detergents present in effluents has irritated the roots by withdrawing the water from them and may contribute for the poor development of fruits and also germination [22].

Biosensors to detect different parameters can be designed to detect the presence of hazardous chemicals, soil pH, microorganism count or their oxygen consumption. Few of the biosensors for environment monitoring are whole cell biosensors detecting toxins where the device can be immobilized with organisms like bacteria, yeast, fungi or plant or animal cells which have multi receptor to bind with the respective compound to produce an electronic signal. This type of biosensor has been used for water monitoring [23]. Disposable DNA probe (Gene chip) is designed by Affymetrix (USA) as lab-on-a-chip assay technology to detect microorganisms contaminating the soil. Measurement of bacterial growth in the soil samples can be used to determine the effect of chemical agents on soil by electrochemical sensors, where the metabolic activity of the organism can be measured amperometrically like Bactometer (Bactomatic INC., Princeton, USA and the Malthus INC., Stoke – on- trent, UK) are few devices designed as biosensors. Optic biosensors (MN, USA) used in medical diagnosis to monitor blood pH and dissolved oxygen and carbon dioxide concentrations can be modified and used for agro- food environmental sector [24]. An amperometric biosensor detecting benzoic acid in some fruit juices and ground water was prepared using a clark type oxygen electrode which was immobilized with a mushroom homogenate where the result obtained was in accordance with AOAC method [25].

Immunobiosensor impregnated with monoclonal antibodies onto indium tin oxide (ITO) electrodes is designed to detect microbial contamination like *Escherichia coli*. On site detectors for rapid and real time monitoring can be created with immunosensors which has wide applications in food industry, water companies and regulatory authorities. Recent development in the production of tailored antibodies as recombinant antibodies, abzymes, plantibodies, MIP-molecular imprinting polymers [26] are synthesized which has specificity to recognize and bind strongly with them. To analyze different environmental pollutants affinity biosensors are designed using different transducers [27], [28].

Nanobiosensors are used to measure quantitatively oxygen consumption in the respiration of microbes either good or bad in the soil. The device consists of microbes placed in between two sensors and immersed into soil sample suspended in buffer solution and the amount of oxygen consumed by the microbes

are detected and quantified. Nano biosensors are designed to detect pests, nutrient content, contaminants and plant stress during drought, temperature or pressure to help farmers to enhance crop production. Different kinds of compounds present in industrial and urban effluents, sewage sludge, landfill leak water, ground water and irrigation water can be detected by the development of biosensors based on photosystem II which can be used to monitor several pollutants [29].

V. CONCLUSION

The results from this study shows that the poor germination and slow development of root, shoot and secondary roots in seeds exposed to different cleaning agents has clearly indicated a reduction in germination percentage and seed vigor index. One of the reason would be the deposition of these harsh chemical containing agents continuously in to the soil. It can change the pH, increase the salinity, change the microbe's composition and their characteristic features, change in the nutrient composition and loss in the fertility of the soil. Continuous use of these agents can adversely affect the soil and directly on the development of seeds and plants. So, design of biosensors to detect and monitor the changes happening in the soil or water used for irrigation can help the farmers to develop healthy plants or crops. The experiment also shows the use of a natural sample that is besan which has showed a remarkable germination in seeds when compared to commercially available cleaning agents. If care in use of products and regular monitoring of the soil can help in better quality and quantity of foods.

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VII. REFERENCES

- [1] G. Grurer, C. Narrod, and L. Abbott, Agriculture, "Food and Water Nanotechnologies for the poor: Opportunities and Constraints", IFPRI Policy Brief, Vol 19, 2011.
- [2] J.D. Newman, A.P.F. Turner, "Biosensors: the analyst's dream, Chemistry and Industry", pp 374-378, 1994.
- [3] A.P.F Turner, I. Karube, S.W. Wilson, "Biosensors, fundamentals and applications", Oxford Science publications, Oxford, 1986.
- [4] E.T. Powner, F. Yalcinkaya, "Intelligent biosensors", Sensor review, 17 (2), pp 107-116, 1997.
- [5] J. Wang, "Glucose biosensors 40 years of advances and challenges", Electroanalysis, 13, pp 983-988, 2001.
- [6] M. Charles and S. Walter, "Ingredients of soap, detergent and shampoo", Department of Natural Sciences/ chemistry, Baruch college, New York, pp 1-67, 2007.
- [7] E. Epstein, J.M. Taylor and R.L. Chamey, "Effect of sewage sludge and sludge compost applied to some soil chemical properties", Journal of Environmental quality, 5, pp 423-426, 1976.



- [8] L.J. Forney, W.T. Liu, J.B. Guckert, Y. Kumagai, E. Namkung, T. Nishihara and R.J. Larson, "Structure of microbial communities in activated sludge, potential implications for assessing the biodegradability of chemicals", *Ecotoxicology and Environmental safety*, 49: pp 40-53, 2001.
- [9] D. Mulkerrins, A.D.W. Dobson, and, E. Colleran, "Parameters affecting biological phosphate removal from waste waters". *Environmental Inter*, 30, PP 249-259, 2004.
- [10] U. J .Strotmann, H. Eglsaer, and U. Pagga, "Development and evaluation of a growth inhibition test with sewage bacteria for assessing bacterial toxicity of chemical compounds" *Chemosphere*, 28, pp 755-766, 1994.
- [11] S. Liu, F. Q. Dong, C.Q. Tang, T.Y. Kuang, L.B. Li, and Y. Liu, "Photodamage to pigment in the photosystem reaction center D1/D2/Cytochrome b559 complex", *Journal of Integrated Plant Biology*, 48 (7), pp 800-806, 2006.
- [12] I. Moya, M. Silvestri, O. Vallon, G. Cinque, and R. Bassi, "Time resolved fluorescence analysis of the photosystem II antenna proteins in detergent micelles and liposomes", *American Chemical Society*, 2001.
- [13] B.N. Ivanov, L.K. Ignatova, and A. K. Romanova, "Diversity in forms and functions of carbonic anhydrase in terrestrial higher plants". *Russian Journal of Plant Physiology*, 54 (2), pp 143-162, 2007.
- [14] D. Tang, R. Jankowiak, M. Seibert, and G. J. Small, "Effects of detergent on the excited state structure and relaxation dynamics of the photosystem II reaction center: A high resolution hole burning study". *Journal of photosynthesis Research*, 27 (1), pp 19-29, 1991.
- [15] A.A.Abdul Baki and J.D. Anderson, "Relationship between decarboxilation of glutamic acid and vigor in soybean seed", *Crop Science*, 13, pp 222-226, 1973.
- [16] N.A. Aizdaicher, Reunova, A. Yu, "Effects of detergents on in vitro growth of diatom alga *thalassiosira pseudonana*", *Russian Journal of Marine Biology*, 28 (5), pp 324-328, 2002.
- [17] L. Nand, and M. Richa, "Synthetic detergent induced changes in the seed inhibition pattern and dehydrogenase activity in mung bean (*Vigna radiata*), *Eco Eny Conservation*, 9 (3), pp 379-383, 2003.
- [18] J. Park, Y. Gu, Y. Lee, Z. Yang, and Y. Lee, "Phosphatidic acid induces leaf cell death in Arabidopsis by activating the rho- related small G protein GTP ase –mediated pathway of reactive oxygen species generation". *Plant physiology*, 134 (1), pp 129-136, 2004.
- [19] K. K .Brandt, M.E. Hesseloy, E.P. Rosloey, K. Enriksen, and J. S. Oyrensen, "Toxic effects of linear alkyl – benzene sulfonate on metabolic activity , growth rate and microcolony formation of nitrosomonas and nitrosospira strains". *Applied Environmental Microbiology*, 67 (6), pp 2489-2498, 2001.
- [20] M. Behzadipou, M. Kluge, and S.Liithjea, "Changes in plasma membrane fluidity of corn (*Zea mays* L) roots after Brij 58 treatment". *Protoplasma*, 217, pp 65-69, 2001.
- [21] Y. A. Reunova, and N. A. Ayzdaycher, "Effects of detergents on chlorophyll a content and quantity dynamics of microalga *Chroomonas salina* (Wils.) Butch (Crypto – phyta)", *International Journal on Algae*, 5, pp 106-110, 2003.
- [22] S.E. Jogerson, "Industrial wastewater management", *Elsevier Scientific Company*, New York, pp 387, 1979.
- [23] I.E Tothill, A.P.F Turner, "Developments in bioassay methods for toxicity testing in water treatment", *Trends in Analytical Chemistry* 15, pp 178-188, 1996.
- [24] S.K Stephens, D.C. Cullen, P.J. Warner, "Novel detection systems for rapid assays in the food industry", *The European Food and Drink Review*, Autumn, pp 83-88, 1997.
- [25] J. Wang, S.A. Kane, J. Liu, M.R. Smyth, K. Rogers, "Mushroom tissue – based biosensor for inhibitor monitoring". *Food Technology and Biotechnology*. 34, pp 51-55, 1996.
- [26] A.P.F Turner, "Immunosensors: The next generation", *Nature Biotechnology* 15 (5), pp 421,1997.
- [27] M.P Marco, S. Gee, B.D. Hammock, "Immunochemical techniques for environmental analysis, 1. Immunosensors". *Trends in Analytical Chemistry*, 14, pp 341-350, 1995.
- [28] S. J .Setford, Es. R.M Van, Y. J .Blankwater, S .Kroger, "Receptor binding protein amperometric affinity sensor for rapid β - lactam quantification in milk". *Analytica Chimica Acta* 398, pp 13-22, 1999.
- [29] M.T Giardi, E.V. Piletska, "Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors and Bio devices". *Biotechnology Intelligence Unit Co Published by Land Biosciences and Springer*, 2006.