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Biofilmed Biofertilizers for Rhizo-Remediation and Consumer Health-Friendly Potato Production

Amila Priyashantha Henagamage[1], Gamini Seneviratne[2], Charmalie Abayasekera[3]

[1]Department of Science and Technology, Faculty of Applied Sciences, UvaWellassa University, Passara Road, Badulla, Sri Lanka
Email: *amila.henagamage1980[at]gmail.com* (Corresponding Author)

[2]National Institute of Fundamental Studies, Hanthana Road, Kandy, Sri Lanka
Email: *gaminis[at]ifs.ac.lk*

[3]Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka
Email: *charmaliea[at]gmail.com*

Abstract: ***Excessive use of chemical fertilizers (CF) in agriculture with soil degradation is associated with risks such as accumulating toxic chemical contaminants in the soil and also edible parts.Two pot experiments were conducted under greenhouse conditions to evaluate rhizo-remediation abilities of different biofilm biofertilizer (BFBF) and CF treatments on nitrosamine and heavy metal contaminants. Potting media were added with dimethyl nitrosamine and selected heavy metal solution mixturesof $CdCl_2$ and $PbCl_2$separately as the test contaminants.Potato plants as the test crop grown in the pots with the contaminants were treated with different combinations of BFBF (a combination of Bacillus pumilus, Bradyrhizobium japonicum, Bacillus subtilis and Trichoderma harzianum) and CF. Rhizo-remediation abilities of the treatments were evaluated by measuring the nitrosamine and heavy metal content in the soil and potato tuber.Results showed that the BFBF applied with 50% of recommended CF rate for potato (50CB) significantly ($P < 0.05$) reduced nitrosamine in tubers and in the potting media. 50CB showed significantly ($P < 0.05$) the lowest tuber and soil Cd and Pb contents in comparison with 100% CF (100C). The findings also confirmed that the soil health can be enhanced by the BFBF through the reduction of soil chemical contaminants.***

Keywords: Biofilmed Biofertilizer; Bioremediation; Heavy metals; Nitrosamine

1. Introduction

In the last five decades, the rate ofCFand agrochemical application has increased tremendously. Although CF has been shown to have beneficial effect on crop yield, incorrect agrotechnical measures and improper fertilization practices can lead to seriously disturbed functions of the entire agroecosystems and contribute to the formation of toxic contaminants like nitroso compounds in the soil. It has been reported that out of numerous contaminants in the soil, nitrosamines deserve special attention as they are included among the most dangerous ecological poisons [3]. Dimethyl nitrosamine is an extremely toxic compound belongs to the nitroso group commonly found in soil in low concentrations [22,17]. They can be formed in agricultural soils treated with pesticides, fungicides like thiram and receiving heavy doses of N fertilizers like urea [2]. N-nitroso compounds are stable in the soil [32] and can be translocated into vegetable crops [28,5]. Intensive application of N fertilizers and agrochemicals is a common practice in cultivations like potato. Since potato tuber is an underground part, there is a great possibility to contaminate the tuber with nitrosamines, as also reported [1, 28].

Moreover, heavy metals are another important group of environmental pollutants threatening the health of human, population and natural ecosystems. Heavy metals can affect the quality of agricultural soils, including phytotoxicity and transfer of heavy metals to the human diet through crop uptake [19].It has been well documented that synthetic fertilizers and pesticides contain trace metals as impurities or active ingredients [16].For example, chemical phosphatic fertilizers such as TSP can contain appreciable amounts of Cd [24] and Cd contamination in soils of many countries is mainly a result of the use of Phosphate (P)[23]. These P fertilizers also introduce other contaminants, such as Hg, As, and Pb, into agricultural soils [26].

Mitigating soil toxicities through microbial interventions, also known as rhizo-remediation is performed by applying beneficial microorganisms, and it is considered as one of the most promising methods to improve soil quality. It has been proven that the rhizo-remediation can be carried out by artificial introduction ofviable microbial populations to contaminated sitesor by stimulating viable native populations. Moreover, microbial biofilms have been found to be suitable for the remediation of pollutants because of their high microbial biomass with exo-polysaccharides and ability to immobilize pollutants[27].Therefore, association of plant growth promoting rhizobacteria could provide plants with extra benefits in terms of rhizo-remediation by removal of most of the soil toxicities [12]. Thus, the current study was focused to investigate the rhizo-remediation ability of the biofilmstowards nitrosamine and heavy metal removal.

2. Materials and Methods

2.1 Pot experiment under greenhouse conditions

Two pot experiments (P1 and P2)were conducted under greenhouse conditions to evaluate the rhizo-remediation abilities of different treatments on nitrosamine and heavy metals, respectively. The experiments were conducted at the Regional Agriculture Research and Development Center, Bandarawela, Sri Lanka.

Riversand (particle size ≤ 1 mm, pH 5-7) was used as the medium for the pot experiment P1,after washing the sands with water followed by heat sterilization to determine the effect of different CF and BFBF treatments on nitrosaminecontamination.The sterilized sand medium was filled into black plastic pots and each pot was amended with diluted nitrosamine solution prepared from3 ppm of dimethyl nitrosamine.Finely ground soil samplesobtained from Regional Agriculture Research and Development Center, Bandarawela, Sri Lanka was used as the medium for the pot experimentP2 to determine the effect of different CF and BFBF treatments on Cd and Pbcontamination. The initial concentrations of available Cd and Pb in the soil were determined (Kisku*et al*., 2011) through acid digestion method (1 perchloric acid: 4 nitric acid)using Atomic Absorption Spectrophotometry (AAS)(Cd-0.3 ppm and Pb-3.5 ppm) and the medium was spiked with solutions having 5 ppm Cd and 25 ppm Pb using $CdCl_2$ and $PbCl_2$. The final available heavy metal concentration of the soil was determined using AAS.

A blend of urea (2.0 g/kg), Triple Super Phosphate (TSP) (3.33 g/kg), and Muriate of Potash (MOP) (1.33 g/kg) was mixed with the pot medium as a basal fertilizer mixture at the time of the seeding. The biofilm culture was diluted by 250 times with clean water before the application and sprayed directly on to the soil and sand mediaof the two experiments, P1 and P2. Disease free seed tubers ('Granola' variety) obtained from Regional Agriculture Research and Development Center, Bandarawela, Sri Lanka (government certified), were grown in each potwith the spiked heavy metal containing soil mixture and the nitrosoamine containing sand mixture. Fertilizer application (2.0 g/kg of urea and 1.33 g/kg of MOP) was repeated after five weeks from the seed sowing,together with the biofilm application. Rates of the CF application was calculated per plant basis according to the DOA recommendations. Moisture level of the medium was maintained constantly by applying 250 ml of water for each pot every day.Plants were grown with a daily minimum-maximum temperature range of 20 ^{o}C – 30 ^{o}C. All the pots were arranged according to CRD inside the greenhouse and treatment combinations were 100% CF (100C), 50% CF (50C), 50% CF + BFBF (50CB), BFBF alone (B), and no amendments (0CB) with five replicates for each treatment.After 90 days from seed sowing, plants were uprooted without damaging the tubers and were washed carefully with water to remove unwanted materials. Subsequently, the tubers were transferred into black polythene bags and were brought in to the laboratory for further analysis. Sand and soil samples were also collected into black polythene bags separately to analyze nitrosamine and heavy metal contents.

2.2 Determination of the availability of nitrosamine contaminants

Rhizo-remediation abilities of different treatments on nitrosamine were evaluated by analyzing the tuber and sand samples separately. Briefly, peels of the tubers were removed carefully up to 5 mm depth from the peripheral surface after washing thoroughly with clean water to remove the direct nitrosamine contaminations. Twenty grams of tuber mass obtained from the peripheral region of the tubers was used to extract nitrosamine with dichloromethane at 55 °C using soxhlet apparatus. Subsequently, the extracts were evaporated using rotary evaporator to concentrate the available nitrosamines. The resulting extracts were analyzed by Fourier transform infrared (FTIR) spectroscopy(Nicolet 6700 FTIR, available at National Institute of Fundamental Studies, Kandy, Sri Lanka) for the availability of nitrosamine. Peak resembles to 1440 cm^{-1}- 1460 cm^{-1} (NNO bond) wavelengths was considered as the responsible peak for nitrosamine detection [18]. Nitrosamine availability of the sand medium was evaluated using the same method as explained above.Statistical data analysiswas performed using one-way Analysis of Variance (ANOVA) Model in MINITAB 16 Statistical Software. The mean values of the absorbance obtained from the FTIR spectroscopy for nitrosamine availability in sand and tuber biomass were compared separately on treatment basis using the Tukey's simultaneous test at 5% probability level.

2.3 Determination of the availability of Pb and Cd as heavy metals

Rhizo-remediation abilities of different treatments on heavy metals were evaluated by analyzing the availability of Pb and Cd in the soil samples and by analyzing the heavy metal uptake in the tuber biomass.Briefly, peels of the tubers were removed carefully up to 5 mm depth from the peripheral surface of the tuber after washing thoroughly with clean water to remove the direct contaminations at the tuber surface. Subsequently, 10 g of chopped tuber samples were measured and air-dried for a day separately, to reduce the water content, followed by oven-drying at 70 °C for 48 hours to constant weight. Preparation of soil samples was performed as explained above.

The dried samples (both tuber and soil samples separately) were ground manually with ceramic mortar and pestle to pass through a 2mm non-metal sieve to ensure uniform particle size. To 1 g of each dry sample, 10 ml of concentrated acid solution (1 perchloric acid: 4 nitric acid) was added and the mixture was allowed to stand overnight, and then heated for 4 hours at 125^{o}C on a hot plate. The hydrolyzed samples were transferred to a centrifuge tube for centrifugation at the rate of 3000 rpm to remove solid particles. The presence of Pb (detection limit, 0.5 ppm) and Cd (detection limit, 0.1 ppm) were analyzed using the Atomic absorption spectrophotometer (AAS Model No. GBC 933AA, Australia) at 217.0 nm and 228.8 nm wavelengths, respectively (standards were $Pb(NO_3)_2$ and $Cd(NO_3)_2$). Samples were analyzed in triplicates.Based on the absorbance data, heavy metal concentrations in the tuber and soil samples were determined. Mean concentrations of the treated soils were compared with the concentration of initial soil [13].Statistical data analyses were performed as explained above.

3. Results and Discussion

3.1 The effect of CF and BFBF treatments on nitrosamine contaminations

Significantly the lowest ($p < 0.05$) sand nitrosamine content (0.0112AU (Absorbance Unit per milliliter) ± 0.001) was

observed in the treatment 50CB(a combination of *Bacillus pumilus, Bradyrhizobium japonicum, Bacillus subtilis* and *Trichoderma harzianum* with 50% CF), except no nitrosamine treatment,0NCB (Figure 1). It is noteworthy that 50CB reduced the nitrosamine content of the sand medium by approximately 77% compared to 100C. It was observed that 100C enhanced nitrosamine content of sand medium by approximately 36% compared to N0CB. Also in tuber biomass, significantlythe lowest ($p < 0.05$) nitrosamine content was detected from the treatment 50CB (0.0015AU± 0.0004). The treatment 50CB reduced the tuber nitrosamine content as much as 95% compared to 100C, and 93% compared to 50C. Further, treatment B (0.0154 AU ± 0.003) also reduced the tuber nitrosamine content compared to 100C and 50C.This experiment further shows that the beneficial microbial community in the biofilm combination BFBF along with or without 50% CF effectively remediate nitrosamine contamination in sand medium. Further, the microbial biomass of the biofilm combination BFBF reduces the penetration of nitrosamine into the tuber mass.

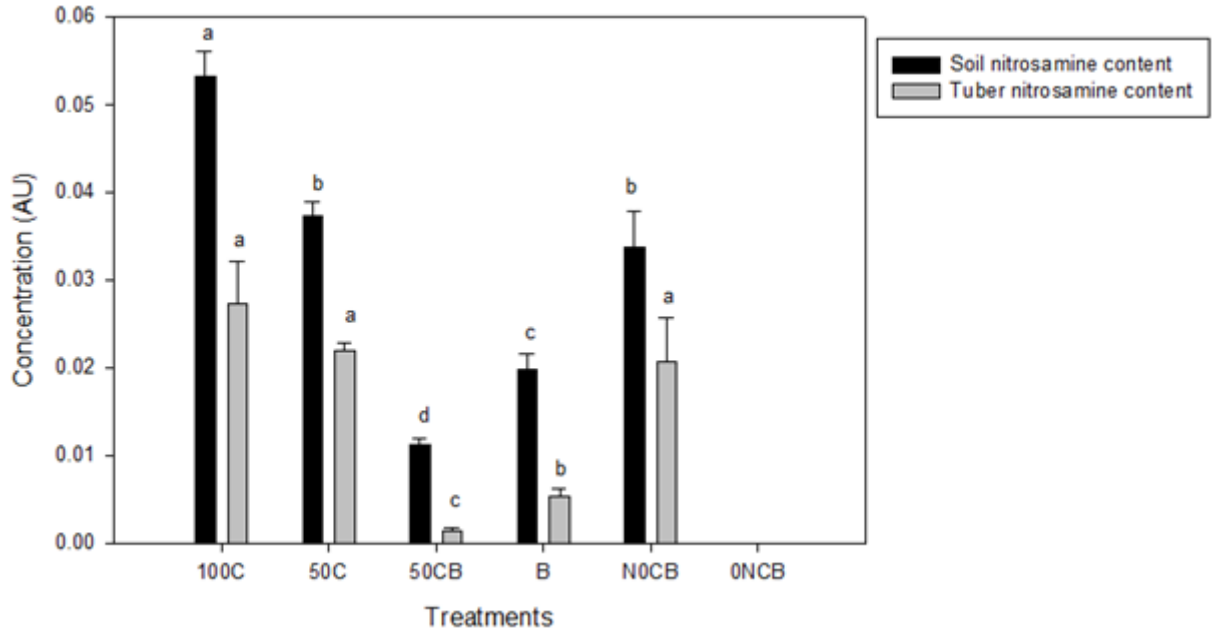

Figure 1: The effect of different CF and BFBFs treatments on nitrosamine content in sand and tuber tissues analyzed by FTIR spectroscopy. Treatments 100C, 50C, 50CB, B, N0CB and 0NCB are 100% CF, 50% CF, 50% CF+ BFBF, BFBF alone, no amendments (including nitrosamine) and no amendments (excluding nitrosamine) treatments, respectively. a, b c, d and cd are letters which indicates the differences in categories. Columns with the same letter are not significantly different at 5% probability level. Vertical bars show standard deviations

Nitrosamine availability in the natural soil is very low [17]and the detection and the quantification are highly impossible using FTIR spectroscopy. Further, soil is a complex medium containing different nitrogenous compounds, which can interfere with FTIR spectroscopic measurements. Therefore, purified sand was used as the medium to avoid the major interferences from other N containing compounds and to obtain clear FTIR spectrum. It has been reported that there is an accumulation of nitrosamines in the soil due to presence of precursor compounds derived from agrochemicals [33] and also excess use of N fertilizers [8]. Natural degradation of nitrosamine contaminants accumulated in the soil is very slow and can persist for extended time periods [31]. However, some soil microorganisms have been reported to degrade nitrosamines to simple compounds in co-metabolic processes, and then use them as nutrients [3]. The data presented herein suggest that the bacterium oxidizes the nitrosamine primarily to *N*-nitrodimethylamine (NTDMA), which is then metabolized further to produce less toxic compounds like *N*-nitromethylamine (NTMA) and formaldehyde [9]. The results of the current study also clearly showed a reduction of sand and tuber nitrosamine content with the application of the biofilm. Bacterial strains *Pseudomonas mendocina* and *Bacillus* sp. have also recently been reported to be capable of metabolizing dimethylnitrosamine [29, 20]. Another study has reported that a variety of monooxygenase expressing bacterial strains are capable of degrading most of the nitrso compound available in the soil [29].

3.2 The effect of CF and BFBF treatments on heavy metal contamination

It was clearly observed that the biofilm treated soil reduced the availability of both Cd and Pb in the soil compared to no biofilm treatment (table 1). The treatment 50CB showed significantly ($P < 0.05$) the lowest soil Cd availability (74% reduction compared to 100C) and the lowest soil Pb availability (34% reduction compared to 100C). Interestingly, treatment B reduced the availability of soil Cd by 60% and soil Pb availability by 33% compared to 100C.Similar trends were observed in the tuber tissues (50CB reduced the tuber Cd availability by 89% and tuber Pb availability by 73% compared to 100C). Therefore, it is clear that all the biofilm treatments significantly reduced the penetration of both soil Cd and Pb content into the tuber tissues.

Table 1: The effect of different CF and BFBFs treatments on heavy metal contaminants in soil and tuber tissues analyzed by AAS

Treatments	Soil Cd (µg/g) Mean±SD	Tuber Cd (µg/g) Mean±SD	Soil Pb (µg/g) Mean±SD	Tuber Pb (µg/g) Mean±SD
100C	5.53^{a}±0.30	0.92^{ab}±0.07	21.96^{b}±1.31	3.93^{a}±0.60
50C	4.95^{ab}±0.21	0.85^{b}±0.06	18.96^{c}±0.74	3.54^{a}±0.81
50CB	1.43^{d}±0.34	0.10^{d}±0.01	14.36^{d}±0.67	1.06^{c}±0.69
B	2.21^{c}±0.73	0.23^{c}±0.02	14.68^{d}±0.51	2.33^{b}±0.39
0CB	4.41^{b}±0.09	0.96^{a}±0.06	20.81^{b}c±1.42	4.00^{a}±0.81
Initial	5.21^{a}±0.11		28.39^{a}±2.31	

Treatments 100C, 50C, 50CB, B and 0CB are 100% CF, 50% CF, 50% CF+ BFBF, BFBF alone and No amendmentstreatments respectively. Initial- soil just after artificial spiking of heavy metals. Values in the same columns with the same letter are not significantly different at 5% probability level.

Generally, heavy metals are impossible to be degraded biologically and persist in the environment for extended periods [11]. Addition of phosphate fertilizers is one of the pathways to accumulate heavy metal contaminants like Cd in the soil [25]. However, soil beneficial microorganisms are able to detoxify soil pollutants that include heavy metals such as Pb [10] and Cd [6, 34]. For instance, a strain of *Pseudomonas maltophilia*has been shown to minimize environmental mobility of toxic ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+}[4,23,11]. Inoculation effects of plant growth promoting rhizo-bacteria *Methylobacteriumoryzae* and *Burkholderia* sp. isolated from rice (*Oryza sativa*) tissues on potato grown in Ni and Cd treated soil has been studied [14]. These bacterial strains have significantly reduced the toxicity of both metals under pot culture conditions [11]. Moreover, bioremediation using microbial biofilms has been considered as one of the promising approaches to reduce the soil heavy metal contaminants[27]. In a study, Cd, Cu, Se and Zn were reported to be biosorbed by *Streptococcus aureus, Bacillus subtilis, Bacillus licheniformis, Pseudomonas aeruginosa* and *Serratia marscecens* in mixtures of Gram positive and negative bacteria [11]. In the current study, it was obvious that the biofilm combinations reduced the level of heavy metals (Cd and Pb) in soil and thereby reducing toxicities accumulating in tuber tissues. There are reports on the application of biofilms for the removal of heavy metals. Recently, bio removal of Cr (III) using bacterial biofilm in a continuous flow reactor has been reported [30]. The ability of a biofilm of *Escherichia coli* supported on Na zeolite for the removal of Cr(VI), Cd(II), Fe(III), Ni(II) from wastewater was also reported [7]. Moreover, rhizo-remediation of certain heavy metals has been studied with the use of *E. coli* biofilms[27].

4. Conclusions

All biofilm treatments reduced the level of nitrosamine and targeted heavy metals (Cd and Pb) availability in the soil and the tuber tissues. Biofilm treatment (a combination of*Bacillus pumilus, Bradyrhizobium japonicum, Bacillus subtilis and Trichoderma harzianum*)along with 50% CF was the best treatment in reducing nitrosamine and heavy metal contaminations in tuber tissues. Therefore, it can be concluded that the biofilm treatment has the ability to remediate nitrosamine, Cd and Pb contaminants in the soil and tuber tissues.

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References

[1] Anon., Nitrosamines found in cutting oils, Toxic Mater. News 3, 156-157, 1976.
[2] Ayanaba, A., Verstraete, W., Alexander, M., Formation of dimethylnitrosamine, a carcinogen and mutagen, in soils treated with nitrogen compounds. *Soil Science Society America Proceedings* 37, 565-568, 1973.
[3] Barabasz, W., Albińska1, D., Jaśkowska, M., Lipiec, J., Fertilization on Soil Microorganisms. *Polish Journal of Environmental Studies* 11, 193-198, 2002.
[4] Brewer, W.S., Draper, A.C., Wey, S.S., The detection of dimethylnitrosamine and diethylnitrosamine in municipal sewage sludge applied to agricultural soils. *Environmental Pollution (Series* B)1 (1), 37-43, 1980.
[5] Casova, K., Cerny, J., Szakova, J., Balík, J., Tlustos, P., Cadmium balance in soils under different fertilization managements including sewage sludge application. *Plant Soil Environment*55(8), 353–361, 2009.
[6] Das, N., Lakshmi, V., Basak, G., Jaseetha, A.S., Abigail, E.A. M., Application of Biofilms on Remediation of Pollutants – An Overview. *Journal of Microbiology and Biotechnology Research*2 (5), 783-790, 2012.
[7] Fishbein, L., Overview of some aspects of occurrence, formation and analysis of nitrosamines. *The Science of the Total Environment* 13, 157-188, 1979.
[8] Fournier, D., Hawari, J., Streger, S. H., McClay, K., Hatzinger, P. B., Biotransformation of N-nitrosodimethylamine by Pseudomonas mendocina KR1. *Applied Environmental Microbiology*72, 6693−6698, 2006.
[9] Harrison, M.J., The arbuscular mycorrhizal symbiosis: an underground association. *Trends in Plant Science*2, 54-60, 1997.
[10] Khan, M.S., Zaidi, A., Wani, P.A., Oves, M., Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*7, 1–19, 2009.
[11] Khan, A.G., Mycotrophy and its significance in wetland ecology and wetland management. In: Wong MH (eds) *Developments in ecosystems*1, 97–114, 2004.
[12] Kisku, G.C., Pandey, P., Singh, M.P., Misra, N.V., Uptake and accumulation of potentially toxic metals (Zn, Cu and Pb) in soils and plants of Durgapur industrial belt *Journal of Environmental Biology*32, 831-838, 2011.
[13] Madhaiyan, M., Poonguzhali, S., Sa, T., Metal tolerating methylotrophic bacteria reduces nickel and

cadmium toxicity and promotes plant growth of tomato (lycopersiconesculentum L.). *Chemosphere*69, 220–228, 2007.

[14] McLaughlin, M.J., Tiller, K.G., Naidu, R., Stevens, D.P., Review: The behavior and environmental impacts of contaminants in fertilizers. *Australian Journal of Soil Research*34, 1 – 54, 1996.

[15] McLaughlin, M. J., Hamon, R. E., McLaren, R. G., Speir, T. W., Rogers, S. L., Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research*, 38, 1037-1086, 2000.

[16] Mhlongo, S.H., Mamba, B.B., Krause, R.W., Nitrosamines: A review on their prevalence as emerging pollutants and potential remediation options. *Water SA*35 (5), 735-740, 2009.

[17] Mistry, B.D., A handbook of spectroscopic data: Chemistry (UV, IR, PMR, CNMR and Mass spectroscopy). Oxford book company, Jaipur, India, 2009.

[18] Nicholson, F.A., Smith, S., Alloway, B.J., Carlton-Smith, C., Chambers, B.J., An inventory of heavy metals inputs to agricultural soils in England and Wales. *Science of the Total Environment*311, 205-219, 2003.

[19] Oliveira, I.M., Bonatto, D., Henriques, J.A.P., Nitroreductases: Enzymes with Environmental, Biotechnological and Clinical Importance. In: Mendez-Vilas A, editor. Current Research, Technology and Education in Applied Microbiology and Microbial Biotechnology, Formatex1008–1019, 2010.

[20] Pancholy, S.K., Formation of carcinogenic nitrosamines in soil. *Soil Biology and Biochemistry*10, 27-32, 1978.

[21] Park, C.H., Keyhan, M., Matin, A., Purification and characterization of chromate reductase in Pseudomonas putida. *General Meeting of American Society for Microbiology*99, 536–548, 1999.

[22] Pierzynski, G.M., Sims, J. T., Vance, F., Soils and Environmental Quality. (2nd ed), CRC Press, LLC,p. 243, 2000.

[23] Premarathna, H.M.P.L., Hettiarachchi, G.M., Indraratne, S.P., Trace Metal Concentration in Crops and Soils Collected from Intensively Cultivated Areas of Sri Lanka. *Pedologist* 54(3), 230-240, 2011.

[24] Premarathana, H.M.P.L., Hettiarachchi, C.M., Indraratne, S.P., Accumulation of Cadmium in Intensive Vegetable Growing soils in the Up Country. *Tropical Agricultural Research*17, 93-103, 2005.

[25] Quintelas,C., Rocha, Z., Silva,B., Fonseca,B., Figueiredo,H., Tavares, T., Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an *E. coli* biofilm supported on kaolin. *Chemical Engineering Journal*149, 319–324, 2009.

[26] Raymond, D. and Alexander, M., Plant uptake and leaching of dimethylnitrosamine. *Nature*,262, 394-395, 1976.

[27] Sharp, J. O., Wood, T. K., Alvarez-Cohen, L., Aerobic biodegradation of N-nitrosodimethylamine (NDMA) by axenic bacterial strains. *Biotechnology and Bioengineering* 89, 608−618, 2005.

[28] Sundar, K., Sadiq, I.M., Mukherjee, A., Chandrasekaran, N., Bioremoval of trivalent chromium using Bacillus biofilms through continuous flow reactor. *Journal of Hazardous Materials*196, 44–51, 2011.

[29] Tate, R. L. and Alexander, M., Stability of nitrosamines in samples of lake water, soil, and sewage. *Journal of National Cancer Institute*54, 327-330, 1975.

[30] Tate, A. and Alexander, M., Resistance of nitrosamines to microbial attack. *Journal of Environmental Quality* 5, 131-133, 1976.

[31] Tesfai, K., Mallik, M.A. B., Pancholy, S.K., Possible formation of a carcinogenic nitrosamine from an insecticide, phosphamidon, and nitrate in soil.*Proceeding of Oklahoma Academy of Science.* Langston University, Langston, Oklahoma 57, 167-168, 1977.

[32] Wang, T., Sun, H., Jiang, C., Mao, H., Zhang, Y., Immobilization of Cd in soil and changes of soil microbial community by bioaugmentation of UV-mutated *Bacillus subtilis* 38 assisted by biostimulation. *European Journal of Soil Biology*65, 62-69, 2014.