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Algae and Their Bacterial Consortia for Soil Bioremediation

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This mini-review summarizes algal applications on soil bioremediation, both for the axenic cell growth to remove hazardous contaminants, and the consortia of bacteria and microalgae/cyanobacteria for organic pollutant degradation. It has been seen that algae and their artificial symbiosis with pollutant-degrading bacteria possess some advantages in the soil bioremediation. Furthermore, development of formulated enzymes from algae and/or bacteria -rather than whole cells- as effective agents for bioremediation will be a novel option in this field.

1. Introduction

With the increase in the anthropogenic activities around the world, soil contamination and remediation of polluted sites have become a worldwide priority with the increasing environmental concerns (Bundschuh et al., 2012; DEA, 2001; Luo et al., 2009; SSR, 2010). In Europe, about 340,000 sites are contaminated, only about 15% of which have been successfully remediated (EEA, 2014). In the United States, office of Solid Waste and Emergency Response (OSWER) has reported the remediation of over 540,000 sites and 23 million acres of contaminated land for anticipated use (Treasury Board of Canada Secretariat, 2014). Similarly, industrial processing, such as petroleum refinery, mineral mining, and chemical manufacturing, and agricultural activities in Australia have caused soil contamination with heavy metals, hydrocarbons, mineral salts, particulates, etc. The total number of contaminated sites was estimated at 80,000 across that country (DECA, 2010). In 2014, the Chinese Ministry of Environmental Protection and the Ministry of Land and Resources have released the first-ever bulletin of a nationwide soil pollution survey, in which it is estimated that nearly 20 million hectares of farmland were contaminated by heavy metals. This may result in a reduction of more than 10 million tons of food supplies each year in China (Wei and Chen, 2001).

Several different factors, such as the physical-chemical properties of the pollutants and how the soil can affect its chemical state, influence the biodegradability of a given pollutant. Diverse soils vary in their ability to overcome pollution, and these differences may be difficult to foresee, for example, because of the behaviour of ionisable organic compounds. There is an enormous interest in developing strategies for remediation of environmental contaminants. However, due to the high costs of physicochemical strategies, the use of biological methods is a more applicable option, using the capability of biological agents as bacteria, fungi, microalgae and higher plants to degrade persistent pollutants. Soil bioremediation is a process that utilizes the metabolic versatility of microorganisms to clean up hazard pollutants. Its ultimate aim is to completely convert organic contaminants to harmless constituents, such as CO_2 and water. It can only be a feasible remedial technology if the microorganisms are capable of acquiring new metabolic pathways and synthesizing appropriate enzymes to achieve the desired degradation within a reasonable period of time.

Cyanobacteria are among the oldest forms of life on the earth, appearing in the fossil record as much as 3.5 billion years ago (Schopf and Packer, 1987). Historically, cyanobacteria have made significant contribution to evolve the earth's atmosphere from being very reducing and anaerobic in the past to being oxidizing in the current time. Microalgae are unicellular microscopic phytoplanktonic species which include cyanobacteria, diatoms, dinoflagellates and green flagellates (Hallegraeff, 2002). They are also ubiquitous and comprise a substantial proportion of microbial biomass in soils. We will hereafter refer both cyanobacteria and microalgae

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to as algae for our further discussion. Algae have been isolated from freshwater, saltwater, and soil. Thermophilic and halophilic variants have also been isolated. Nitrogen-fixing forms of algae act as natural fertilizers for rice and other crops. They are motile, and exhibit chemotaxis and phototaxis. In comparison to the sessile higher plants, algae have faster growth rates and capacities to colonize many surfaces, which attenuate competition for resources. Algae are currently responsible for ~50% of O_2 genesis and CO_2 sequestration globally. They play an important role in the carbon balance for the entire world. A few species are able to degrade polycyclic aromatic hydrocarbons (PAHs) and other constituents of crude oil and refined petroleum products (Cerniglia, 1992; Chueng and Kinkle, 2001). Others have potential applications in CO_2 capture and remediation (Stephens et al., 2010).

This mini-review article is not intended to provide a thorough survey of the numerous microbial remediation strategies for recovery of the polluted lands. Instead, we try to illustrate microalgae-based technologies for soil bioremediation in the form of both axenic cell growth and consortia with contaminant-degrading bacteria for the removal of environmental pollutants.

2. Algae for the detection of soil pollution

Algae are often used as indicators to evaluate the ecotoxicity, genotoxicity and environmental risk of pollutants, both in soil and sediments due to their sensitivity to the presence of toxic chemicals (Subashchandrabose, et al., 2013; Tigini et al, 2011). Given the sensitivity of algae to pollutants, variation in the algal species composition can serve as a useful bioindicator of pollution, and should be used in conjunction with bioassays and chemical analysis for toxicological estimations of samples from various habitat (Trevors,1984). An illustrative example is that the intracellular dehydrogenase and urease (important enzyme in nitrogen metabolism) activity is a common method of estimating the total microbial activity in soils and they are very sensitive to the presence of various pollutants (Megharaj et al. 1994, 1999).

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) has been widely used throughout the world to control arthropod disease-vectors and agricultural pests. As a result, DDT residues have now become persistent environmental contaminants. Megharaj et al. (Megharaj, Kantachote, et al. 2000) examined the impact of DDT contamination towards soil algae, microbial biomass, and activity of a soil enzyme (dehydrogenase). They found that viable counts of bacteria and algae declined with the increasing DDT contamination while fungal counts, microbial biomass and dehydrogenase activity increased in medium-level contaminated soil (27 mg DDT residues kg-1 soil). All the tested parameters were greatly inhibited in high-level contaminated soil (34 mg DDT residues kg-1 soil). Thus, long term DDT contamination exerts a negative influence on soil biological properties, as manifested by the observed decrease in populations of different microflora, their biomass and enzyme activity. Species composition of algae and cyanobacteria was altered in contaminated soils and sensitive species were eliminated in the medium and high contaminated soils. The observation suggested that these organisms could be useful as bioindicators of pollution.

3. Algal Degradation of PAHs

PAHs are a group of chemicals that contain two or more fused aromatic rings in linear, angular or cluster arrangements. Physical and chemical properties of PAHs vary with the number of rings and hence their molecular mass (Cerniglia, 1992, Cheung and Kinkle, 2001). PAH resistance to oxidation, reduction and vaporization increases with molecular weight, whereas the aqueous solubility of these compounds decreases. As a result, PAHs differ in their behaviour, distribution in the environment, and their effects on biological systems. The EPA has identified 16 PAHs as priority pollutants (Manoli and Samara, 1999). Some of these PAHs are considered to be possible or probable human carcinogens, and hence their distribution in the environment and possible exposure to humans has been the focus of much attention. (Menzie et al, 1992). Especially, high-molecular weight PAHs are important constituents of petroleum as they are recalcitrant pollutants (Chueng and Kinkle, 2001). PAHs are relatively stable and recalcitrant in soil and less easy to degrade than many other organic compounds. They are difficult to remove from contaminated soil using other treatments.

PAHs biodegradation has been tested (Lei et al. 2007) using microalgae *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda* and *Raphidocolis captricornutum* on fluoranthene and pyrene. The removal of these compounds was specific to algal species and was also toxicant-dependent. PAHs degradation in 7-days of treatment was 78% in the case of *S. capricornutum* (the most effective species) and 48% with *C. vulgaris* the least efficient species. Fluoranthene removal efficiency was higher than pyrene for all species, except for *S. platydiscus*. The degradation efficiency of fluoranthene and pyrene in a mixture was comparable, or higher than the respective single compound, suggesting that the presence of one PAH stimulated the degradation of the other PAH.

4. Algal Bioremediation of Petroleum Contaminated Soil

One of the major causes of pollution is the dispersion in the environment of petroleum oil and petrochemical products. Some studies on the influence of total petroleum hydrocarbons (TPH) contamination on microalgae and microbial activities in a long-term contaminated soil was carried out (Megharaj, Singleton, et al. 2000). Microbial biomass, soil enzyme activity, and microalgae declined in medium to high-level (5,200–21,430 mg kg^{-1} soil) TPH-polluted soils, whereas low-level pollution (2,120 mg kg^{-1} soil) stimulated the algal populations and showed no effect on microbial biomass and on the content of enzyme activity. It is worth mentioning that measurement of intracellular dehydrogenase and urease (an important enzyme in nitrogen metabolism) activity is a common method for estimating the total microbial activity in soils (Trevors 1984) and they are very sensitive to various pollutants (Megharaj, Boul, and Thiele 1999). Interestingly, inhibition of all the tested parameters was more marked in soil considered to have medium-level pollution than in soils that were highly polluted.

5. Algal Bioremediation of Pesticides/Fertilizers

Algae may be utilized as soil conditioners, or as biofertilizers (Metting, 1981). Many microalgae have shown significant advantages over bacteria and fungi in degrading organic pollutants. In particular, they can overcome the necessity of carbon and other nutrients and can contribute to sequestration of CO_2. However, research on the degradation of organic pollutants by algae, especially on the capabilities of mixotrophs, lags far behind that of bacteria (Subashchandrabose et al. 2011). Algae form an important component of soil microflora and are ubiquitous, probably accounting for up to 27% of the total microbial biomass in the soil (McCann, Annette E and Cullimore 1979). These microorganisms are involved in maintaining soil fertility and oxygen production. They are more widespread than the other free-living microorganisms capable of dinitrogen fixation (Burns, Richard C. Hardy 1975) and thus are very important for the nitrogen economy of soils.

Several reports on biodegradation of pesticides by algae and cyanobacteria are available in the literature (Megharaj, Kantachote, et al. 2000; Megharaj, Venkateswarlu, and Rao 1987; Megharaj et al. 1994). El-Bestawy et al. (El-Bestawy, El-Salam, and Mansy 2007) reported that the cyanobacterial strains *Synechococcus, Oscilatoria*, *Nostoc, Nodularia*, and *Cyanothece* were able to degrade the pesticide lindane at a very fast rate. Kuritz and Wolk (Kuritz and Wolk 1995) have also tested the capacity of filamentous cyanobacteria (*Anabaena* sp. and *Nostoc ellipsosporum*) to degrade lindane even though this organism was genetically engineered to degrade 4-chlorobenzoate, which is another pollutant. Both unicellular green algae *C. vulgaris* and *S. bijugatus* and cyanobacteria *S. elongates, P. tenue* and *N. linckia* were able to metabolise monocrotophos and quinalphos, two organophosphorus insecticides with equal potential (Megharaj, Venkateswarlu, and Rao 1987). Also, Megharaj et al. (Megharaj et al. 1994) have demonstrated the potential of two green unicellular algae *C. vulgaris* and *S. bijugatus*, and four cyanobacteria *N. linckia, N. muscorum, O.animalis*, and *P. foveolarum* in the degradation of methyl parathion, a structurally closely related organophosphorus pesticide to fenamiphos, by cellular metabolism. Furthermore, Megharaj et al. (Megharaj, 2000) showed that the cyanobacterium *N. muscorumwas* was able to completely degrade methyl parathion, including its hydrolysis to product p-nitrophenol. It was also found that *Scenedesmus* sp. MM1, *Chlamydomonas* sp., and *Nostoc* sp. MM2 were able to accumulate appreciable quantities of the parent compound fenamiphos and its primary oxidation product FSO. The degradation of the organophosphorus pesticide, fenamiphos (O-ethyl-O3-methyl-4-methylthiophenyl]-iso-propylamidophosphate), was shown by individual species of five green algae (*Scenedesmus* sp. MM1, *Scenedesmus* sp MM2, *Chlamydomonas* sp., *Stichococcus* sp., *Chlorella* sp., and five cyanobacteria (*Nostoc* sp. MM1, *Nostoc* sp. MM2, *Nostoc* sp. MM3|, *Nostoc muscorum*, *Anabaena* sp.). Fenamiphos sulfone phenol, FSOP, and FSO were detected in the culture extracts of these algae and cyanobacteria. These reports highlighted the ability of the above algae and cyanobacteria to detoxify pesticides. It has opened the road to consider algae as valuable microorganisms for bioremediation of some pesticides and their toxic metabolic products (Cáceres, Megharaj, and Naidu 2008).

Megharaj et al. (Megharaj, Boul, and Thiele 1999) have first reported on the effects of DDT and its metabolites on soil algae and enzymatic activities in a spiked soil under laboratory incubation conditions. The authors highlighted that the replacement of sensitive species by resistant ones in contaminated soils, such as replacement of nitrogen-fixing cyanobacteria by green algae could result in a decrease in biodiversity and loss of important ecological functions. Thus, the altered biological properties in the contaminated soils could potentially lead to loss of soil fertility. In this study, five pure cultures, two unicellular green algae (one *Chlorococcum* sp. isolated from high-level DDT-contaminated soil, and the other from uncontaminated soil) and three species of cyanobacteria (*Anabaena* sp. isolated from the low-level contaminated soil of the present study and two species of *Nostoc* from a pentachlorophenol (PCP)-polluted soil) were tested for their ability to metabolize DDT and its main metabolites (Megharaj, et al. 2000).

6. Consortia of Algae and Bacteria

Microalgae and cyanobacteria associate with other aerobic or anaerobic microorganisms to form microbial groups living symbiotically in a community defined consortia. The consortia of algae and bacteria can work in a synergistic way for the detoxification of organic and inorganic pollutants, compared to the individual microorganisms. Since the photosynthetic pathway for algae can only be utilized in the shadow layers of soils, there may exist some limitation for the algal bioremediation of contaminated environments in the deeper layers where light does not arrive. The use of microalgae and bacteria jointly can thus be complementary and synergic for a better pollutant degradation efficiency. On one hand, algal photosynthesis produces oxygen, a key electron acceptor for the pollutant-degrading heterotrophic bacteria to degrade organic matter. On the other hand, bacteria provides carbon dioxide and other stimulatory means to support the photoautotrophic growth of their partners (Subashchandrabose et al, 2011). The self-oxygenation of this natural systems which have already been tested are advantageously exploited for remediation of many pollutants (Muñoz and Guieysse, 2006). In fact, the biodegradation processes by means of algae-bacteria consortia are an ideal self-sustaining system, that is cheaper and technically superior compared to conventional engineering technologies, which have several disadvantages as higher costs for oxygen supply, incomplete utilization of natural resources, creation of secondary pollutants, and technical impracticability in some situations (Subashchandrabose et al. 2013). Mix of different strains (e.g., algae–bacteria) might have a synergistic effect and the microbial populations can perform functions that are difficult or even impossible for individual strains or species (Escobar et al., 2008). Some advantages of the co-culture are robustness to environmental fluctuations, stability for the members, ability to survive to periods of nutrient limitations, ability to share metabolites and resistance to invasion by other species. In addition, microalgae are found to enrich toxic chemicals on their cell-wall, forming a surface zone that facilitate enhanced degradation by bacteria (Luo, et al., 2014).

Biotechnological exploitation of algal and bacterial consortia requires a deeper understanding about the microbial interactions and organization from the ancient stromatolites and modern cyanobacteria mats. Thus, modern molecular techniques and selection of the desired microbial consortium members will allow engineering of self-sustaining systems with dual mission of pollutant removal and metabolite production. In nature, there are many evidences of microbial communities of cyanobacteria or microalgae and bacteria, either fossilized or living together. The potential of cyanobacteria/microalgae–bacterial consortia as the self-sustained system for (a) detoxification of environmental pollutants and removal of nutrients, and (b) production of metabolites/by-products of huge commercial value, coupled with the mitigation of greenhouse gas CO_2 has been deeply reviewed by Subashchandrabose et al. (Subashchandrabose et al. 2011). Therein a detailed list of examples where cyanobacteria/microalgae and bacteria consortia have been tested in the bioremediation of organic and metal pollutants has been provided.

Negatively-charged polysaccharides and carbohydrates containing amino, carboxyl, hydroxyl or sulfide groups are the main constituents of cell walls of microalgae and cyanobacteria. This feature allow metals binding through the negatively-charged ligand groups, which is the basis for metal removal from wastewaters and, potentially, from soil. Moreover, metal adsorption can occur also by uptake into cells. In fact, incorporation into vacuoles or aragonite ($CaCO_3$) structures, precipitation on the cell surface or internally can occur. Even though, heavy metals are potent inhibitors of photosynthesis as they can replace or block the prosthetic metal atoms in the active sites of certain enzymes, the acidic functional groups of bacterial cell walls can also bind significant concentrations of aqueous cations, which can affect the speciation, distribution and mobility of those cations (Ginn and Fein, 2008). Therefore, algae growing in wastewater may provide a simple, long-term strategy for removal of metal pollutants.

7. Conclusions

In comparison to other microbial treatment, algal soil bioremediation overcome the necessity of carbon and other nutrients. It may provide the additional benefits of renewable energy generation by algal CO_2 fixation. Algal–bacterial consortia have shown to be more efficient for the treatment of hazardous pollutants. The mechanism for cell metabolism underlying the synergistic degradation of organic pollutants in the soil needs to be further studied with computational biology and experimental biology so that the interaction between algae and bacteria may be quantified at both molecular level and metabolic level. It should be noticed that in contrast to the wastewater treatment in which the recovery of the pollutants transformed to any kind of biomass is quite easy and feasible, it is almost impossible in soil (with the only exception of phytoremediation). Research on this aspect should be encouraged.

As a matter of fact, the capability to degrade chemicals depends on the enzymatic patrimony of the biological species. Contrary to microorganisms that need particular conditions to grow, isolated enzymes can readily work for the degradation of the pollutants. This feature has led to develop formulated enzymes -rather than whole cells- as agents for bioremediation.

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References

Bundschuh, J., Litter, M.I., Parvez, F., Román-Ross, G., Nicolli, H.B., Jean, J., Liu, C., López, D., Armienta, M.A., Guilherme, L.R.G., Cuevas, A.G., Cornejo, L., Cumbal, L., Toujaguez, R., 2012. One Century of Arsenic Exposure in Latin America: A Review of History and Occurrence from 14 Countries, *Sci. of the Total Environ.*, 429, 2–35

Burns, R.C., Hardy, R.W.F., 1975. *Nitrogen Fixation in Bacteria and Higher Plants*. Springer.

Cáceres, T.P., Megharaj, M. Naidu, R., 2008. Biodegradation of the Pesticide Fenamiphos by Ten Different Species of Green Algae and Cyanobacteria. *Current Microbiology* 57 (6), 643–46.

Cerniglia, C.E., 1992. Biodegradation of polycyclic aromatic hydrocarbons, *Biodegradation*, Kluwer Academic Publishers.3, 351-368.

Cheung P.Y., Kinkle, B.K., 2001. Mycobacterium Diversity and Pyrene Mineralization in Petroleum-Contaminated Soils. *Appl. Environ. Microbiol.* 67(5),2222-2229

Department of the Environment, Australia (DEA), 2001. Land Theme Report, http://www.environment.gov.au/node/21774#totalimmobile.

Department of Environment and Conservation, Australia (DECA), 2010, Assessment Levels for Soil, Sediment and Water.

European Environment Agency (EEA), 2014. Progress in Management of Contaminated Sites, 2014, http://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment.

El-Bestawy, E., El-Salam, Z., Mansy, E.R.H., 2007. Potential Use of Environmental Cyanobacterial Species in Bioremediation of Lindane-Contaminated Effluents. *International Biodeterioration and Biodegradation* 59,180–92.

Escobar, J., Brenner, M., Whitmore, T.J., Kenney, W.F., Curtis, J.H., 2008. Ecology of testate amoebae (thecamoebians) in subtropical Florida lakes. *Journal of Paleolimnology.* 40(2), 715-731

Ginn, B.R., Fein, J.B., 2008. The effect of species diversity on metal adsorption onto bacteria. *Geochimica et Cosmochimica Acta* 72, 3939–3948.

Kuritz, T., Wolk, C.P., 1995. Use of Filamentous Cyanobacteria for Biodegradation of Organic Pollutants. *Applied and Environmental Microbiology* 61 (1), 234–38.

Lei, A.P., Hu, Z.L., Wong, Y.S., Tam, N.F.Y., 2007. Removal of Fluoranthene and Pyrene by Different Microalgal Species. *Bioresource Technology* 98 (2), 273–80.

Luo, Y., Wu, L., Liu, L., Han, C., Li, Z., 2009, Heavy Metal Contamination and Remediation in Asian Agricultural Land, *National Institute of Agro-Environmental Sciences*, MARCO Symposium, Japan, October 5-7.

Luo, S., Chen, B., Lin, L., Wang, X., Tam, N.F.Y., Luan, T., 2014. Pyrene Degradation Accelerated by Constructed Consortium of Bacterium and Microalga: Effects of Degradation Products on the Microalgal Growth. *Environmental Science & Technology*, 48, 13917−13924

Manoli, E., Samara, C., 1999. Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. *TrAC Trends in Analytical Chemistry*, 18(6), 417–428

Marshall, J. A., Nichols, P.D., Hallegraeff. G.M., 2002. Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae. *Journal of Applied Phycology*. 14(4),255-265

McCann, A.E., Cullimore, D.R., 1979. Influence of Pesticides on the Soil Algal Flora. in *Residue Reviews*, ed., Gunther, J.D., Gunther, F.A., Residues o, 1–31. Springer.

Megharaj, M., Venkateswarlu, K., Rao, A.S.,1987. Metabolism of monocrotophos and quinalphos by algae isolated from soil, *Bulletin of Environmental Contamination and Toxicology*, 39(2), 251-256

Megharaj, M., Madhavi, D.R., Sreenivasulu, C., Umamaheswari, A., Venkateswarlu, K., 1994. Biodegradation of Methyl Parathion by Soil Isolates of Microalgae and Cyanobacteria. *Bulletin of Environmental Contamination and Toxicology* 53, 292–297.

Megharaj, M., Boul, H.L., Thiele, J.H., 1999. Effects of DDT and Its Metabolites on Soil Algae and Enzymatic Activity. *Biology and Fertility of Soils* 29 (2), 130–34.

Megharaj, M., Kantachote, D., Singleton, I., Naidu, R., 2000. Effects of Long-Term Contamination of DDT on Soil Microflora with Special Reference to Soil Algae and Algal Transformation of DDT. *Environmental Pollution* 109, 35–42.

Megharaj, M., Singleton, I., McClure, N.C., Naidu, R., 2000. Influence of Petroleum Hydrocarbon Contamination on Microalgae and Microbial Activities in a Long-Term Contaminated Soil. *Archives of Environmental Contamination and Toxicology*.

Megharaj, M., Venkateswarlu, K., Rao, S., 1987. Metabolism of Monocrotophos and Quinalphos by Algae Isolated from Soil. *Bulletin of Environmental Contamination and Toxicology* 39, 251–56.

Menzie, C.A., Potocki, B.B., Santodonato, J., 1992. Exposure to carcinogenic PAHs in the environment. Environ. Sci. Technol., 26 (7), 1278–1284.

Metting, B., 1981. The systematics and ecology of soil algae. *The Botanical Review.* Springer. 47(2):195-312

Munoz, R., Guieysse, B., 2006. Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water research*, 40(15), 2799–2815

Schopf, T.M., Packer, B.M., 1987. Early atchean (3.3-billion to 3.5-billion-yea-old) microfossils from Warsanoona group, Australia. *Science* 237, 70-73.

Shift Soil Remediation (SSR), 2010, Soil Contamination in West Africa,
http://www.scribd.com/doc/71599035/Soil-Contamination-in-West-Africa

Stephens, E., Ross, I.L., Mussgnug, J. H., Wagner, L.D., Borowitzka, M.A., Posten, C., Kruse, O., 2010. Future prospects of microalgal biofuel production systems. *Triends in Plant Science*. 15(10):554–564.

Subashchandrabose, S.R., Balasubramanian R., Megharaj, M., Venkateswarlu, K., Naidu, R., 2011. Consortia of Cyanobacteria/microalgae and Bacteria: Biotechnological Potential. *Biotechnology Advances.* 29(6), 896–907.

Subashchandrabose S.R., Ramakrishnan B., Megharaj M., Venkateswarlu K., Naidu R., 2013. Mixotrophic Cyanobacteria and Microalgae as Distinctive Biological Agents for Organic Pollutant Degradation. *Environment International*.

Tigini, V., Giansanti, P., Mangiavillano, A., Pannocchiam, A., Varese, G.C., 2011. Evaluation of toxicity, genotoxicity and environmental risk of simulated textile and tannery wastewaters with a battery of biotests. Ecotoxicol Environ Saf., 74(4), 866-873.

Treasury Board of Canada Secretariat, 2014, Contaminants and Media, http://www.tbs-sct.gc.ca/fcsi-rscf/cm-eng.aspx?qid=1200518.

Trevors, J.T., 1984. Effect of Substrate Concentration, Inorganic Nitrogen, O2 Concentration, Temperature and pH on Dehydrogenase Activity in Soil. *Plant and Soil.* 77, 285–93.

Wei, C.Y., Chen, T.B., 2001. Hyperaccumulators and phytoremediation of heavy metal contaminated soil: a review of studies in China and abroad. *Acta Ecologica Sinica* 21, 1196-1203.