

TURNING WASTE INTO A RESOURCE FOR REMEDICATION OF CONTAMINATED SOIL IN TROPICAL DEVELOPING COUNTRIES

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ABSTRACT

Contaminated soil from industrial or agricultural activities poses a health threat to animals and humans and can also have a detrimental effect on economic systems by making land unsuitable for agriculture and other economic purposes. This problem is of particular concern in tropical developing countries where agriculture is the economic base. Traditional methods for soil remediation are often expensive and energy consuming. In-situ bioremediation has been proposed as a cheaper alternative to conventional methods in areas where remediation would otherwise not be implemented. Despite encouraging results in the laboratory, the practice of in-situ bioremediation is limited, partially due to its inefficiency at low temperatures. The objective of this study is to provide an inventory of some waste products that potentially can be used as amendments for in-situ bioremediation in developing countries in tropical climate. Emphasis has been given to map efficient methods that are appropriate to economically marginalised people in such countries. Waste from livestock operations, crop residues and processing waste constitute the major waste flows in many developing countries. A number of organic by-products can potentially be used to stimulate microbial activity for bioremediation purposes. Three amendments; whey, pyroligneous acid and compost teas were selected to be studied in detail due to their liquid nature and documented capacity to stimulate microorganisms with capacity to degrade pollutants. Experiments are needed to determine their potential for in-situ bioremediation in developing countries in tropical climate.

KEYWORDS

Bioremediation, Organochlorine pesticides, Polycyclic aromatic hydrocarbons, Milk whey, Pyroligneous acid, Compost teas.

1 INTRODUCTION

The environmental sustainability of the human society largely depends on our management of the natural environment and the ecosystems that constitute the platform upon which our civilization is based. Yet almost two-thirds of the world's ecosystems are considered degraded as a result of damage, mismanagement and failure to look after these resources [1]. Contaminated soil from industrial or agricultural activities poses a potential health threat to animals and humans and can have a detrimental effect on economic systems by making land unsuitable for agriculture and other economic purposes.

Waste management programs in developing countries are often deficient and the inappropriate disposal of waste is an increasing problem worldwide [2]. Furthermore the use of pesticides and other toxic products is minimally restricted in many developing countries. Being labour saving and relatively cheap [3], many toxic products are widely used which leads to extensive contamination with persistent substances such as polycyclic aromatic hydrocarbons (PAH) and organochlorine pesticides [2,4]. Traditional methods for soil remediation are often expensive and energy consuming and the elevated costs involved in removal of toxic substances from contaminated soils prevent remediation from being carried out; especially in areas of little economic value [5]. *Bioremediation*, i.e. the utilisation of microorganisms and plants with the ability to degrade or immobilize toxic compounds has been proposed as a cheaper alternative to conventional methods [4-6]. Despite encouraging results in the laboratory [7-10], the practice of in-situ bioremediation is limited [11,12], partially due to its inefficiency at low soil temperatures [8]. Soil temperatures in tropical countries however present conditions that are favourable for in-situ soil bioremediation. In-situ bioremediation is thus potentially an appropriate technology for tropical regions, especially in contaminated areas of little economic value where remediation would not be implemented with more expensive methods.

Agriculture is the economic base of most tropical developing countries, accounting for 50 % of the employment [13]. Organic by-products from agriculture, forestry, fishery and livestock operations are available in abundance and are typically of limited economical value [14]. Many of these by-products could potentially be used as carbon sources to stimulate microorganisms to degrade toxic compounds.

The objective of this study is to provide an inventory of some waste products that potentially can be used as amendments for in-situ bioremediation in developing countries in tropical climate.

2 INVENTORY METHODOLOGY

A literature review was undertaken to identify suitable carbon sources with potential for bioremediation that are available in tropical countries. Emphasis was given to map low cost/low technology methods that are appropriate to economically marginalised people in developing countries. A number of criteria were selected for the inventory and the organic waste flow in tropical developing countries was screened for amendments that complied with the listed criteria;

2.1 Physical criteria of the amendments

- Carbon source readily available for microorganisms
- Source of macro and micronutrient

- Presence of relevant biodegrading microorganisms or capacity to stimulate the growth of such organisms.

2.2 Availability and economic criteria

- Significantly cheaper than conventional methods
- Low technology/low requirement for special skills
- Being a by-product of little economic value.

In this study, Nicaragua was used as a case to determine the feasibility of the amendments. Due to the lack of scientific data available for some of the amendments in Nicaragua, informal sources were used in addition to scientific reports.

3 RESULTS

The economy of most developing countries in tropical climate is based on agriculture, livestock, fishing and forestry [13,15,16]. Such operations generate a number of by-products of little economic value that frequently create serious environmental problems when disposed of. Animal feed has been proposed as an outlet for some of these by-products but due to low levels of protein, high levels of moisture and some anti-nutritional factors i.e. presence of tannins and polyphenols, this practice is limited [17,18]. However being inexpensive and easily available sources of carbon, nutrients and bioactive compounds [19], many of these by-products can potentially be used as sources of readily available carbon for bioremediation purposes [20].

3.1 The inventory

According to an inventory that identified the major agricultural by-products in Costa Rica; abattoir waste and manure from different livestock constituted the greatest waste flows together with crop residues and processing waste of bananas, sugarcane, oil palm, cocoa, coffee, rice, fruits etc. [16]. Meat, poultry and fish industries, followed by fruit and vegetable processing industry produce the highest loads of food waste on a global scale [21] suggesting that the results from Costa Rica would be similar in Nicaragua and other tropical developing countries. Whey is another important organic residual product that is often disposed of directly to the environment [22]. Furthermore seafood processing plants discard large amounts of fish tissue as by-product materials [23]. Tropical carbon sources from the forest industry include saw dust, bark, pyroligneous acid (PA) from charcoal production etc. [14,20,24]. Additional suitable by-products exist that are common only in particular regions [25]. In Table 1 a list of tropical organic by-products complying with the criteria listed above is presented.

Experiments have demonstrated that some of the listed amendments have the ability to enhance the degradation of different toxic compounds [12,20,26]. Lignocellulosic wastes such as corn cobs, sugarcane bagasse and sawdust have been shown to enhance degradation of many contaminants including PAH and organochlorines [20,26-28]. Animal wastes and crop residues such as coffee pulp and molasses have proved to enhance degradation of toxic compounds either alone or as ingredients in compost [20,21]. Other by-products have not yet been tried for their bioremediation capacity but their proven capacity to stimulate microbial activity makes them potential candidates for bioremediation purposes [24,29-31].

Type of waste	Examples	Availability/price	Experimental data on bioremediation capacity
Abattoir waste	Bone meal, blood meal	Can be purchased from abattoirs or obtained free of charge at smaller butchery units.	Blood meal [32]
Manures	Manure from cattle, swine, poultry etc.	Typically available free of charge.	Pig manure [33]
Residues from fishery	Fish hydrolysate, fish meal	Low value by-product [23].	Fish bone meal [34,35]
Dairy industry waste	Whey, whey powder,	Typically available free of charge.	Whey [7,9,10]
Lignocellulosic residues, etc.	crop Sugarcane bagasse, straw, corn cobs, rice husks	Typically available free of charge.	Bagasse [27,28] Straw [36] Corn cobs [37] Ricehusks [38]
Non-lignocellulosic residues	crop Molasses, banana waste, coffee pulp, fruits	Molasses can be purchased for 0,35 USD/L. Other crop residues are typically available free of charge.	Molasses [39]
Un-processed residues	wood Sawdust, wood chips, bark	Typically available free of charge at sawmills or carpenters.	Sawdust [27]
Processed residues from wood industry	from Pyroligneous acid, paper mill discards	Pyroligneous acid can be purchased in Nicaragua for 0,75 USD/L [40]	Not found

Table 1. Some organic by-products available in tropical developing countries with potential for bioremediation and their availability in Nicaragua

3.1.1 Analysis of the appropriateness of the amendments in developing countries in tropical climate.

The potential amendments listed in Table 1 all comply with the physical criteria discussed in section 2 and are readily available in most parts of Nicaragua. Being by-products of little economic value, they can typically be acquired free of charge. The economic criteria however, depend not solely on the feedstock itself but on the technologies required to prepare and distribute the amendments. A major limitation for in-situ bioremediation of many of the solid amendments is the vast quantities required for effective bioremediation [20,21]. For instance, good soil colonization by several fungal species was observed with application of corncobs at ratios of 4:1 corn cob to soil [37]. The great volumes needed may lead to considerable costs in terms of labour, transports and machinery. The low mobility of solid amendments compared to liquids can be an obstacle for efficient biodegradation since the amendments are not easily mixed with contaminated subsoil. Liquid amendments have a higher mobility and can thus be expected to more efficiently reach deeper soil layers. The practice to produce aqueous extracts of compost, known as compost teas (CT) has been proposed as an approach to upgrade the value of compostable by-products and avoid handling of bulky material [30,41].

PA is a liquid by-product from the charcoal production whose stimulating effect on the soil microorganisms has received little attention by the scientific community. To the best

knowledge of the authors, PA and CT have not been assessed scientifically for their bioremediation potential. Being liquid amendments with documented stimulating effect on microorganisms with capacity to degrade pollutants and due to the little or inexistent data concerning bioremediation potential of PA and CT, these two amendments were selected to be studied in further detail. Whey is another liquid amendment, available in abundance in Nicaragua that is of special interest since it has previously been studied in our research group. Including whey in further studies would facilitate benchmarking against earlier results and provide information on potential differences in performance between tropical and temperate climate. Table 2 gives an overview of three liquid amendments that could be suitable for soil bioremediation purposes.

	Necessary investment in machinery etc.	Estimated application rates and frequency
Whey	Irrigation equipment and storage tank according to the size of the application area.	Biweekly doses of 32 000 Lha ⁻¹ milk have been used in in-situ experiment [8] and continuous applications of 50 000 Lha ⁻¹ has been reported beneficial in agriculture fields [42].
Compost Tea	Irrigation equipment and storage tank according to the size of the application area Commercial ACT brewers of 20 L capacity cost between 130-200 USD. DIY versions for about 30 dollar exist. Brewers with a capacity of 100 L cost about 1650- 2000 USD [43-45]. NCT can be made in the storage tank.	Application frequency of CT has not been systematically examined [46] but 150-200 Lha ⁻¹ of ACT as soil drench has been recommended by Ingham [47] for agriculture pest control. Considerably higher doses have been used in most experiments [30,31,48,49] and applications of 1700 Lha ⁻¹ at 3 weeks intervals have been proposed to improve mineralisation of organic soil matter [50].
Pyroligneous Acid	Irrigation equipment and storage tank according to the size of the application area Used oil barrel, firewood and metal tube at a cost of 15 USD [51].	Applications of 5-40 Lha ⁻¹ diluted in water have been recommended for control of agriculture pests [40].

Table 2. Characteristics of three selected amendments

3.1.2 Whey

Whey is produced when milk casein is removed from the milk in dairy operations to make cheese and other products. This liquid by-product constitutes between 85-95% of the milk volume and 55% of milk nutrients remain in the whey. About half of the yearly global production of 145 million tonnes is utilized for animal feed etc. The remaining large volumes end up as potential contaminants in the environment [52]. The chemical content of whey is characterised by lactose, a number of essential and non-essential amino acids in different proportions, vitamin B 1,2,6,7,12, folic acid and lactic acid [10].

Relatively few studies have been performed on the effect of whey on biodegradation of organic pollutants [53]. However, research at Mid Sweden University has showed that the degradation of aliphatic and aromatic hydrocarbons can be significantly enhanced by the addition of whey and fermented whey [7,9]. The biodegradation-enhancing effect of whey was primarily attributed to an increased microbial biomass stimulated by the readily available carbon source [10]. The degradation studies also showed a more complex dependence of carbon sources and growth factors, such as B-vitamins, on the degradation of an aromatic compound (phenanthrene) compared to an aliphatic compound (hexadecane) [7]. These results indicate that the presence of co-factors such as vitamins and micronutrients may be

important to consider when evaluating the suitability of organic amendments for bioremediation of soil contaminated by various types of organic pollutants.

The results of the whey method have not been as promising in in-situ experiments as in the laboratory, presumably due to low soil temperature and low availability of the whey due to restriction of percolation in clay soil [8]. In tropical climate the mineralisation rate is expected to be significantly higher and the method could prove more appropriate in such climate if the obstacle of low whey availability can be surpassed. High concentrations of whey has been reported to inhibit biodegradation in some circumstances, and too low concentrations showed little effect [53] suggesting that determination of optimum application rates is important to obtain an efficient degradation. Optimum application rates might vary however, due to factors such as soil type, precipitation, temperature etc. Repeated applications rather than just on one occasion was reported to increase the degradation of a number of organic pollutants [53].

3.1.3 Pyroligneous acid

PA is a by-product of charcoal production [24] with a limited economic value that is also referred to as wood vinegar, pyrolysis oil, smoke extract, bio-oil etc. [54,55]. Currently the only commercially important application of PA is that of smoke flavour in food [54]. Smoke flavour is considered to be safe by the U.S. Food and Drug Administration and can be used at levels that comply with good manufacturing practice [56]. PA is typically disposed of as waste [55] but in Japan it has been used for centuries to increase crop production and to combat agricultural pests [24,57]. At present, the majority of the charcoal consumed worldwide is produced in traditional kilns in developing countries [58]. Such charcoal production lacks appropriate control of the gas and vapours produced in the process and causes land and air pollution [59]. Production systems exist however that recover these condensate co-products or use them as fuel in the pyrolysis process, reducing the emission to a minimum [54]. Several low tech kilns that recover the PA from the charcoal production have been developed including *the advanced Brazilian beehive kiln* [60] and the *Casamance kiln* [61]. In recent years the research and technology concerning pyroligneous acid have advanced significantly [54], and the current trend in charcoal production is to improve the environmental performance while maintaining or improving charcoal yield and quality [62].

The chemical composition of PA varies depending on factors such as type of feedstock, pyrolysis temperature and duration etc. [25,54]. The most frequently identified compounds in PA include methanol, acetic acid, methyl acetone, acetaldehyde, furan, furfural and volatile organic acids [57]. PA contains phenolic compounds that are known to have antimicrobial properties [63] and PA has successfully been used to control fungal growth on wood [64] and bacterial decay of foods [63,65]. At low concentrations however, PA has been shown to stimulate germination, growth and yield in a wide range of plants [66]. Although the growth promoting effect of PA is not yet clearly understood it has been attributed to nutritious components such as nitrates of Ca, Mg, K, magnesium sulphate etc. but also to its stimulatory effect on symbiotic fungi and bacteria [55]. PA has been shown to significantly increase basal respiration and microbial biomass in highly weathered tropical soils suggesting that microbes used PA for their metabolism [24]. Another report [67] reveals that all compounds typically found in smoke emitted from charcoal kilns can be metabolized by strains of phototrophic soil bacteria and that the addition of these compounds clearly intensifies respiratory metabolism in the soil. The formation of fruit bodies of edible fungi such as *Lentinus edodes*, *Pholiota nameko* and *Pleurotus ostreatus* was significantly increased after applications of PA to the growth medium [29]. *Lentinus edodes* and *Pleurotus ostreatus* are known to be versatile degraders of many toxic compounds including PAH and organochlorine pesticides [68]. In a

laboratory experiment PA showed the greatest growth stimulating effect of *Pleurotus ostreatus* at concentrations of 3.0%. At higher concentration the effect was declining and at 10% no fruit-bodies were produced [29].

Microorganisms capable of degrading the compounds found in PA are typically not abundant in soil. However in habitats with a permanent flow of these compounds e.g. soil near a charcoal kiln, their presence has been reported to be increased [67]. Continuous applications of PA could consequently be expected to increase the population of the desired microorganisms. Experimental works with PA against agriculture pests has been implemented since 2005 in a number of regions of Nicaragua and the technology has been well received by local farmers in different regions [51].

3.1.4 Compost teas

CT are liquid compost extracts obtained when compost is soaked in water for a determined number of days. Optionally additives (derived from by-products) such as molasses, fish hydrolysate, rock dust, yeast extract, humic acids etc. are used to add nutrients and stimulate microbial activity. Historical evidence suggest that CT were used in agriculture by Romans and ancient Egyptians [69] but the practice declined as synthetic pesticides became available and at present it is predominantly used in organic agriculture [31]. Anecdotal evidence of disease-suppressive and soil-amending properties abounds but more than often these testimonies lack supporting data. Scheuerell and Mahaffee [46] indicate that the understanding of CT is in its infancy but an increasing number of scientific reports throughout the last decade have confirmed its ability to suppress a wide range of both air- and soil-borne plant pathogens.

A number of production parameters (e.g. aeration, compost source, nutrient additives etc.) have been manipulated in order to optimize plant disease suppression [31]. To date, consensus on optimum production parameters for disease suppression hasn't been reached and some studies report inconsistency in the performance [41,70]. However a number of reports have shown that aeration and additions of nutrients lead to significant increases of the cell mass of active bacteria [30,31,49]. The addition of humic acids and yeast extracts has also proved to significantly increase the fungal populations [49].

Most research so far has focused on non-aerated compost teas (NCT) but the habit to aerate the compost tea in order to maintain aerobic conditions during the extraction process is gaining popularity. During the last decades a number of companies have emerged that manufacture machines for making compost teas under highly aerated conditions. The oxygen concentration in the tea has obvious consequences for the microbial communities and a distinction between aerated compost teas (ACT) and (NCT) is necessary.

To date virtually all research on CT has focused on its potential to control plant diseases in agriculture. However the fact that NCT and ACT have proved to significantly increase soil microbial respiration and dehydrogenase activity [30] makes it an interesting candidate for bioremediation. Plate counts and microscopic examination studies have revealed that ACT has bacterial population dominated by *Bacillus* sp., *Lactobacillus* sp., *Micrococcus luteus*, *Staphylococcus sciuri*, *Pseudomonas putida*, *Burkholderia glumae* and *Clavibacter agropyri* while species of *Aspergillus*, *Penicillium* and *Trichoderma* dominated the fungal communities [49]. Many of these organisms have the capacity to degrade a number of toxic substances including PAH and organochlorine compounds. [12]. Aeration and addition of nutrients was shown to significantly increase the population of the mentioned organisms [49]. To date most published studies have relied on traditional culturing methods or microscopic examination to assess the microbial composition of ACT. These methods provide limited knowledge about

the microbial diversity [41] suggesting that molecular methods are needed determine the microbial diversity and abundance of ACTs.

In natural ecosystems complex symbioses are responsible for the degradation of complex organic structures such as wood, i.e. fungi that break down recalcitrant compounds such as lignin whose metabolites subsequently are broken down by other fungi or bacteria [26]. The diversity and abundance of microorganisms with capacity for bioremediation in nutrient enriched ACT makes it an interesting candidate for bioremediation purposes. The introduction of microbial strains to treat contaminated soil or water is known as bioaugmentation. One of the greatest challenges with bioaugmentation is to assure that the introduced organisms survive the competition with indigenous organisms. The survival time of an introduced organism rarely surpasses a few days or weeks due to predation, lack of or competition for nutrients or chemical attack by other organisms [71] suggesting that frequent soil drenches of ACT are necessary to increase the chances that the target organisms survive.

4 DISCUSSION

Whey, PA and CT are three potential soil amendments for enhancing in-situ bioremediation which comply with the criteria discussed in section 2. Their liquid nature and documented capacity to stimulate microorganisms with capacity to degrade pollutants make them interesting candidates for bioremediation. Experiments are needed to determine their effect on degradation rates and degree of mineralization of organic pollutants in tropical climate. In addition their optimum application rates for bioremediation need to be investigated.

Although the feedstock is virtually free of charge, expenses related to the dissemination of the amendments may become a considerable limitation if great quantities are needed. While Ingham [47] recommends CT applications of 150-200 Lha⁻¹ for agriculture pest control, laboratory tests suggest that considerably higher quantities of CT [30,31,48,49] and whey [7,9,53] are required for efficient bioremediation. With application rates of several tonnes per ha⁻¹ heavy machinery might be needed for large scale operation which could significantly reduce the comparative advantage to solid amendments as compost etc. ACT is considerably more expensive to produce than the other amendments and comparative studies are needed to examine whether the additional cost of aeration is economically justified. The low cost and energy input associated with NCT [46] together with simpler production methods can have a favourable effect on its adoption rate. Most contaminated sites present an indigenous culture of microorganisms capable of degradation the toxic substance [72]. Inclusion of soil from contaminated site in the compost teas could increase its efficiency. It could also be beneficial to add cultures of organisms known to degrade a wide range of toxic compounds such as *Trichoderma harzianum* [26].

The inhibitory effect of PA on microbial activity must be taken into account when application rates are assessed, considering that the contaminant itself may present an additional inhibitory effect. PA is currently not being produced on a large scale in Nicaragua but the traditional charcoal production systems could be upgraded to reduce emissions and recover the PA. An increased demand of PA would be an economic incentive to modify the production system in such direction [54].

Non-technical criteria, i.e. regulatory factors etc. can limit the use of the proposed amendments [5] and such criteria also need to be addressed in future research.

5 CONCLUSIONS

A number of organic by-products can potentially be used as amendments for bioremediation of contaminated soils in developing countries with tropical climate. Three amendments; whey, pyroligneous acid and compost teas were selected to be studied in detail due to their liquid nature and documented capacity to stimulate microorganisms with capacity to degrade pollutants. Experiments are needed to determine their potential for in-situ bioremediation in developing countries in tropical climate.

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