



**BIOAVAILABILITY, TOXICITY AND MICROBIAL VOLATILISATION
OF ARSENIC IN SOILS FROM CATTLE DIP SITES**

**Master of Agricultural Science
at
Adelaide University
Faculty of Agricultural and Natural Resource Science**

By

BAGUS BINA EDVANTORO

**DEPARTMENT OF SOIL AND WATER
WAITE AGRICULTURAL RESEARCH INSTITUTE
GLEND OSMOND, SA
AUSTRALIA**

2000

TABLE OF CONTENTS

ABSTRACT	vi
STATEMENT	ix
ACKNOWLEDGEMENT	x
LIST OF FIGURES	xi
LIST OF TABLES	xiv
CHAPTER 1	1
General introduction	1
CHAPTER 2	5
Literature review	5
2.1. Cattle dip sites in New South Wales (NSW)	5
2.1.1. Current problems	5
2.1.2. Removal of contaminated dip soils	8
2.2. Organochlorine (DDT) pesticides	10
2.3. Arsenical pesticides	12
2.4. Arsenic chemistry	13
2.5. Arsenic behaviour	15
2.6. Effects of arsenic on organisms	17
2.6.1. Man and animals	17
2.6.2. Plants	19
2.7. Arsenic-microbe interactions	21
2.7.1. Microbial resistance to arsenic	21
2.7.2. Biotransformation of arsenic	22
2.7.2.1. Oxidation of As	23
2.7.2.2. Reduction of As	25
2.7.2.3. Methylation of As	26
1. Bacterial methylation of As	27
2. Fungal methylation of As	29
3. Algal methylation of As	32

2.8. Overview of bioremediation	34
2.9. Use of microbes to enhance As contaminated soils	36
2.10. Factors affecting As volatilisation by microbes	37
2.10.1. Arsenic forms	37
2.10.2. Bioavailability	38
2.10.3. Presence of As methylating microbes	40
2.10.4. Presence of toxic substance	41
2.10.5. Environmental conditions	42
1. Soil pH	42
2. Oxygen	43
3. Nutrients	44
4. Soil moisture	45
2.11. Summary	46
CHAPTER 3	48
General materials and methods	48
3.1. Soils	48
3.1.1. Sample collection	48
3.1.2. Samples pretreatment	49
3.2. Analytical procedures	49
3.2.1. Soil physical and chemical analysis	49
3.2.1.1. Soil properties	49
3.2.1.2. Dry weight and field capacity	50
3.2.1.3. Arsenic analysis	51
1. Reagents for As determination	51
2. Sample preparation for total As	52
3. Sample preparation for available As	52
4. Flame atomic absorption spectrometer (FAAS)	53
a. Nitrous oxide-FAAS	53
b. Hydride generation-FAAS	54
3.2.1.4. DDT analysis	55

3.2.2. Microbiological analysis	56
3.2.2.1. Enumeration of soil microorganisms	56
3.2.2.2. Soil respiration (CO ₂ evolution)	56
3.2.2.3. Microbial biomass C	57
3.2.2.4. Microbial population resistant to As and DDT	57
3.2.3. As volatilisation by microbes	58
3.2.3.1. Microbial volatilisation of As (biostimulation)	58
3.2.3.2. Microbial volatilisation of As (bioaugmentation)	61
1. Observation of As methylating fungi	61
a. Preparation of fungal cultures for As methylation	61
b. Preparation of As trapping filter	61
c. Technique for trapping volatile As	62
d. Determination of volatile As	62
e. Identification of fungal strains	62
2. Observation of As methylating algae	63
3.2.3.3. Determination of As volatilisation from soil	64
3.2.3.4. Soil augmentation by As methylating microbes	66
1. Fungal augmentation	66
2. Algal augmentation	67
3.2.3.5. Statistical analysis	68
1. Effect of long-term contamination by As and DDT towards soil microorganisms	68
2. Formation of volatile As compounds through microbial methylation	68
CHAPTER 4	69
Effect of long-term contamination by As and DDT towards soil microorganisms at disused cattle dip sites	69
4.1. Introduction	69
4.2. Results	70
4.2.1. Soil properties	70

4.2.2. Soil As and DDT	70
4.2.3. Soil microbial properties	72
4.2.4. Microbial populations resistant to As and DDT	75
4.3. Discussion	78
4.3.1. Soil properties	78
4.3.2. Microbiological analysis	80
4.3.3. Microbial populations resistant to As and DDT	83
4.3. Summary	85
CHAPTER 5	87
Formation of volatile arsenic compounds through microbial methylation in dip site soils	87
5.1. Introduction	87
5.2. Results	88
5.2.1. Microbial volatilisation of As (biostimulation)	88
5.2.2. Microbial volatilisation of As (bioaugmentation)	92
5.2.2.1. Augmentation by fungi	92
1. Screening of arsine formation by fungi	92
2. Identification of fungal isolates	92
2.a) Fungal culture 1 (identified as <i>Penicillium</i> sp) .	94
2.b) Fungal culture 2 (identified as <i>Ulocladium</i> sp) .	95
3. As volatilisation from soil after fungal augmentation	96
5.2.2.2. Augmentation by algae	97
1. Observation of arsine formation by algae	97
2. As volatilisation by algal augmentation	98
5.3. Discussion	99
5.3.1. Stimulation of microbial As evolution from soils by addition of nutrients	99
5.3.2. Microbial volatilisation of As (bioaugmentation)	104
5.3.2.1. Fungal volatilisation of As	104
5.3.2.2. Algal volatilisation of As	107

5.4. Summary	111
CHAPTER 6	112
Summary and conclusions	112
References	116

Abstract

Arsenic (As) and DDT have been intensively used in the dipping liquid to control cattle tick (*Boophilus microplus*) in northern New South Wales, Australia. Concentrations of total As and hexane extractable DDT in the surface (0-10 cm) soils from 11 dip sites ranged from 34 to 2941 mg As kg⁻¹ and 2.9-7673.2 mg DDT kg⁻¹ soils, respectively.

High residual levels of As and DDT from such cattle dipping operations may have adverse impact on soil microbes which are important for maintaining soil fertility and in assisting soil remediation. Long-term effects of mixed As and DDT contaminants upon soil microbial properties were examined by comparing polluted and unpolluted soils. Microbial studies included the measurement of bacterial and fungal populations as well as microbial biomass C and soil respiration. There was a highly significant difference between the microbial properties of polluted and unpolluted sites ($p \leq 0.001$). In comparison to unpolluted soils, fungal counts, microbial biomass C and respiration were dramatically reduced ($p \leq 0.05$) in polluted soils. Generally, however the bacterial populations between polluted and unpolluted soils were not different ($p \leq 0.05$). The combined effects of As and DDT contaminants resulted in an increased stress on soil microorganisms than a single compound. The results of this study suggest that long-term contamination by As and DDT of soils adjacent to former cattle dipping soils adversely affects soil microbial properties and alters the microbial characteristics as shown by a reduction in fungal abundance and development of selected resistant bacterial population.

Microbial conversion of As plays an essential role in the distribution and mobilisation of As in soils and these mechanisms may remove As from polluted soils. Recently, it has

been reported that microbial methylation of As with subsequent As volatilisation could potentially be developed as a remediation strategy for As contaminated soils. Many studies have investigated As volatilisation by microbes in As polluted soils, but no work has examined this microbial transformation in soils containing additional contaminants of DDT. This study was conducted to assess whether the addition of exogenous nutrients and augmentation of arsenic (As) methylating organisms were able to accelerate the rates of As volatilisation in soils containing mixed contaminants of As and DDT. Results showed that the rates of As loss in long-term contaminated dip soils was stimulated by cow manure amendments and basic environmental optimisation that favours aerobic microbial processes. A minor rate of As loss was observed in control soils either in autoclaved or unautoclaved (without nutrient addition) soils, indicating the process was mediated predominantly by microorganisms. Increasing manure levels added resulted in a greater amount of As release in contaminated soils, following the order: 30% > 15% > 5% (w/w) of manure > cow urine amendment. Soil moisture affected the rates of As loss ($p \leq 0.05$) and the yield was optimised at 75% of field capacity. The supplement of 30% (w/w) of manure at 75% of field capacity soils exerted the greatest reduction of As concentrations (8.3% loss of initial total As concentration) in a contaminated dip soil containing 1390 mg As kg⁻¹ and 194 mg DDT kg⁻¹ in 5 months. The rates of As loss and microbial respiration (CO₂ production) were correlated with added nutrient levels ($p \leq 0.05$).

Screening of As and DDT resistant fungi for As methylating ability shows that 2 fungal isolates (*Penicillium* and *Ulocladium* sp.) were the most active arsine producers yielding 0.32 and 0.40 µg of arsine, respectively in 7 days. These 2 fungal cultures were observed to be able to grow on the contaminated environment of dip soils as indicated by

distribution of fungal mycelia on the entire surface soil in the flask. The augmentation of both fungi enhanced the arsine evolution rates either in field contaminated soils or freshly As-added soils. The amounts of arsine dissipated in contaminated soils and As-spiked soils were 3.7 and 8.3 fold respectively when compared to uninoculated soils. Moreover, an algal species (*Stichococcus* sp.) isolated from As contaminated dip soils was shown to be capable of generating arsine on various As levels substrates. The algal culture produced arsine on the media amended with 25, 50 and 100 $\mu\text{g As mL}^{-1}$ which yielded 0.08, 0.14 and 0.11 μg of arsine, respectively over 7 days. No arsine was trapped on the media added with 200 $\mu\text{g As mL}^{-1}$. The inoculation of this alga to either polluted or As-amended soils was able to accelerate the rates of As volatilisation. Similar to fungal observation, a greater arsine evolution rate was noticed in uncontaminated soils (spiked with 50 mg As kg^{-1}) than in polluted soils. The results suggest that the presence of DDT in contaminated dip soils possibly limits the As volatilisation rates by microbes.

Statement

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution. To the best of the author's knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in Adelaide University library, being available for photocopying and loan.

DATE: 07/05/2001

SIGNED:

Acknowledgement

I would like to thank deeply my supervisors, Drs. Ravi Naidu, Megharaj Mallavarapu, Graham Merrington and Ian Singleton for their enthusiasm, excellent advice, constructive criticism and generosity during the period of my study.

Special gratitude is forwarded to Dr. Michael Priest from NSW Agriculture and Dr. Eileen Scott from Crop Protection Department of Adelaide University for assisting the identification of fungal cultures. I am also grateful to Julie Smith, Lester Smith and Collin Rivers for the technical helps and supports in conducting the laboratory experiment.

Special thanks are addressed to my office, The Environmental Impact Management Agency (BAPEDAL) for giving me a chance and funding to achieve my Master degree in Australia.

There were many other people in the Soil and Water Department of Adelaide University, who kindly helped me in completing my study, particularly Dr. Rob Murray as my academic advisor, John Davey, Tracy Parish and Helen Taylor, I give you my heartfelt thanks. Thanks are also addressed to my fellow students, particularly Yenni, Saidy, Rina, Pak Agus, Nathan, Irda, Sarah and Gerry who constantly provided encouragement during all my work in Adelaide.

Finally, I would like to thank my wife, parents, sisters and brother for their patience, understanding, love and supports in achieving my higher education in Australia.

Lists of Figures

Figure 2.1.	An example of diagrammatic representation of a cattle dip site (after McDougall, 1997)	6
Figure 2.2.	Formulae of arsenical pesticides (after Hiltbold, 1975)	13
Figure 2.3.	Diagram of Eh-pH effects on the As forms at 25°C and with a total As activity of 10 ⁻⁴ M (after Masscheleyn et al., 1991)	15
Figure 2.4.	Chemical forms of As and their transformations in soils (after Bhumbra And Keefer, 1994)	23
Figure 2.5.	Anaerobic methylation pathway for dimethylarsine formation by <i>Methanobacterium</i> sp. (after McBride & Wolfe, 1971)	28
Figure 2.6.	Biosynthetic pathway of trimethylarsine formation by <i>Scopulariopsis brevicaulis</i> (after Tamaki & Frankenberger, 1992)	30
Figure 2.7.	The dissolved metals processes in soil (after Sahut et al., 1994)	39
Figure 3.1.	Schematic diagram of arsine trapping apparatus (after Gao & Burau, 1997)	66
Figure 4.1.	Comparisons of microbial populations between uncontaminated and contaminated soils at selected dip sites including (A) bacterial populations and (B) fungal populations (means ± 1.S.E.). Column with different letters are significantly different to one another	73
Figure 4.2.	Comparisons of microbial properties between uncontaminated and contaminated soils at selected dip sites including (A) microbial biomass C and (B) soil respiration (means ± 1.S.E.). Column with different letters	

	are significantly different to one another	74
Figure 4.3.	Enumeration of resistant fungal populations at selected dip sites on potato dextrose agar media supplemented with varying concentrations of As, DDT and combined As and DDT (means \pm 1. S.E.)	76
Figure 4.4.	Enumeration of resistant bacterial populations at selected dip sites on nutrient agar media supplemented with varying concentrations of As, DDT and combined As and DDT (means \pm 1. S.E.)	77
Figure 5.1.	Total As loss in 2 contaminated soils under varying nutrient and moisture levels. (A) soil E at 35% of field capacity. (B) soil E at 75% of field capacity, (C) soil J at 35% of field capacity. (D) soil J at 75% of field capacity (means \pm 1. S.E)	90
Figure 5.2.	Soil respiration levels as affected by nutrient addition to soil E and J. (A) soil E at 35% of field capacity. (B) soil E at 75% of field capacity, (C) soil J at 35% of field capacity. (D) soil J at 75% of field capacity (means \pm 1. S.E)	91
Figure 5.3.	Morphology of <i>Penicillium</i> sp. as an active arsine producing fungi after staining with toluidine blue in 7 days old. Bars = 50 μ m	94
Figure 5.4.	Morphology of <i>Ulocladium</i> sp. as an active arsine producing fungi after staining with toluidine blue in 7 days old. Bars = 50 μ m	95
Figure 5.5.	Cumulative arsine evolution in soil augmented with 2 fungal species (<i>Ulocladium</i> and <i>Penicillium</i> sp.) (means \pm 1. S.E)	97

Figure 5.6	Cumulative arsine evolution in soil augmented with an algal species	
	(<i>Stichococcus</i> sp.) (means \pm 1. S.E)	99

List of Tables

Table 2.1.	Chemicals used in cattle tick dips (after McDougall, 1997)	7
Table 2.2.	The toxic dose for rates of arsenical pesticides (after Peoples, 1975)	18
Table 2.3.	Methylation of As by bacteria (after Cullen & Reimer, 1989)	29
Table 2.4.	Production of methylated As compounds from different As substrates by yeast and fungi (after Cullen & Reimer, 1989)	32
Table 2.5.	Summary of remediation alternatives in cattle dip sites (after Van Zwieten & Grieve, 1995)	35
Table 3.1.	Treatment of As volatilisation study by biostimulation in 2 contaminated dip soils	60
Table 4.1.	Some physical and chemical properties of contaminated and uncontaminated dip soils (means \pm 1. S.E)	71
Table 5.1.	Screening result of arsine formation by fungi in 7 days incubation	93
Table 5.2.	Algal formation of arsine at various levels of As in 7 days incubation	98