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Interactive effects of PAHs and heavy metal mixtures on oxidative stress in *Chlorella* sp. MM3 as determined by artificial neural network and genetic algorithm

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ABSTRACT

Mixture toxicity studies are very complex due to the complexity exhibited by the chemicals involved, and the net interaction effects are highly dependent on mixture combinations, exposure dose and the test organism. For assessing the toxicity of mixtures, factorial analysis has been widely used, while the usage of models developed by artificial neural network (ANN) analysis and genetic algorithm (GA) is very limited. We combined for the first time the factorial design experiment with ANN and GA to develop a model for predicting the interactive toxicological effects using a soil microalga, *Chlorella* sp. MM3. The chemicals included in the mixtures were two polyaromatic hydrocarbons (PAHs), phenanthrene and benzo[*a*]pyrene, and two heavy metals (HMs), cadmium and lead. Three biochemicals implicated in oxidative stress, viz., malondialdehyde (a measure for lipid peroxidation, LPO), catalase activity and proline accumulation were used as the toxicity criteria. Validation of the predicted results related to the biochemicals with the experimental data clearly indicated that the model developed with the combination of ANN and GA is greatly effective in predicting the toxicity of PAHs and HMs mixtures toward microalga with <10% relative error. Both catalase and LPO were found to be the promising biomarkers for predicting microalgal toxicity of PAHs and HMs mixtures. In addition, a significant positive correlation was evident between the removal of PAHs/uptake of HMs and LPO.

1. Introduction

Availability of information on the toxic effects of pollutants toward living organisms is mandatory for the environmental risk assessment process. In the real scenario, nontarget organisms are exposed to diverse chemicals, and most of the toxicity studies are concerned with either a single chemical or a mixture of organic/inorganic chemical(s). The occurrence of polyaromatic hydrocarbons (PAHs) and heavy metals (HMs), as mixtures, in the urban environment is very well established [1]. In particular, transition HMs are co-contaminants in most of the PAH-contaminated industrial sites [2]. In addition, automobile emission, former gas works sites and smelting industries remain the sources of PAHs and HMs co-contamination [3]. PAHs are released into the environment by both natural and anthropogenic activities, including forest fire, incomplete combustion of fossil fuels, etc. [2,4]. PAHs are toxic to microalgae as they are accumulated into their lipid bodies and affect the photosystem; however, microalgae degrade PAHs as they possess enzymatic system similar to bacteria [5,6]. Although toxicity studies included mixtures of either PAHs [7] or HMs mixtures [8], there are limited investigations that involved mixtures of both PAHs and HMs [9,10].

Factorial design experiments were widely used for detecting non-additive interaction effect of chemical mixtures without knowing their synergistic, additive or antagonistic character [11]. Artificial neural network (ANN), one of the effective artificial intelligence approaches [12] and a nonparametric modeling algorithm that emulates biological neurons in order to understand the relationship between the input patterns and their targets [13], is appropriate for both qualitative and quantitative analysis in pattern recognitions wherein complicated, noisy and imprecise input patterns occur [14]. On the other hand, genetic algorithm (GA) whose optimization principle relies on the biological theory of survival of the fittest that explains how genes on the chromosome vary by several biological means such as chromosome encoding and decoding, selection for offspring, crossover and mutation to adapt to the particular environment has been widely used for search and optimization of the problem [15]. As such, no toxicity studies involved the factorial design

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Table 1
Coded and uncoded levels of the factors.

Factor	Coded low level	Uncoded low level (μg L^{-1})	Coded high level	Uncoded high level (μg L^{-1})
Phenanthrene	−1	50	+1	5000
BaP	−1	50	+1	5000
Cadmium	−1	50	+1	5000
Lead	−1	50	+1	5000

in combination with ANN and GA to determine the nontarget effects of PAHs and HMs mixtures on biochemical changes largely implicated in oxidative stress. For the first time, we conducted a 2^4 full factorial design experiment involving four factors such as phenanthrene (Phe), benzo[*a*]pyrene (BaP), lead (Pb) and cadmium (Cd), at two levels of 0.05 and 5 mg L^{-1}, and three toxicity responses including lipid peroxidation, catalase activity and proline accumulation in a microalga, *Chlorella* sp. MM3, isolated from PAHs-contaminated soil. The choice of the test organism in the present study is in view of the fact that soil microalgae are the better indicators of pollution caused by organic/inorganic chemicals alone or in mixtures [5,6], and PAHs and HMs are known to induce the formation of reactive oxygen species in microalgae in response to oxidative stress [16,17].

2. Materials and methods

2.1. Microalgal strain and growth medium

A soil microalga, *Chlorella* sp. MM3, was isolated from a PAHs-contaminated site in Adelaide, Australia. Axenic culture of the microalga was maintained in 250 mL flasks containing 100 mL of Bold's basal medium (BBM) under continuous illumination of cool white fluorescence light [18]. The culture was checked for contamination periodically under microscope and by streaking on BBM and nutrient agar medium. Logarithmically-growing four-day-old culture was used as inoculum in all the experiments. In toxicity studies, the modified BBM contained one-tenth of the original phosphate and no EDTA. Our preliminary results indicated that >75% of free Cd and 70% of free Pb, as measured with ion-selective electrode and Visual Minteq models as shown earlier [19], are available in the modified medium.

2.2. Chemicals and stocks

Stock solutions of Phe and BaP were prepared in dimethyl formamide. Based on our preliminary studies, it was ensured that volume of the solvent added to the medium to get the final concentration of a PAH had no observable effect on the microalga. Lead nitrate and cadmium nitrate were dissolved in ultrapure water, and the aqueous solutions were sterilized using 0.25 μm disposable filters.

2.3. Toxicity experiments

The algal exposure to chemicals was carried out in five batches of 32 experiments with 160 experimental runs, one batch each for a biochemical and other two for determining PAHs degradation and HMs uptake. After spiking 10 mL medium, contained in 40 mL glass tubes, with the test chemicals as per the factorial design, microalgal inoculum was added at a density of 3×10^5 cells mL^{-1}. The tubes were incubated for 96 h at 25 °C under cool fluorescent white light in an orbital shaker at 140 rpm.

Table 2
Uncoded design matrix for factor combination and the responses measured.

Expt. run	Factor (μg L^{-1})				Response			PAH removal (%)		Metal uptake (ng L^{-1})	
	Phe	BaP	Cd	Pb	MDA	Catalase	Proline	Phe	BaP	Cd	Pb
1	50	50	50	50	6.36	4.23	1.63	100	99.76	0.16	8.62
2	50	5000	5000	5000	13.41	6.95	4.41	99.03	17.90	1.21	115.5
3	5000	50	5000	5000	12.63	7.54	3.72	58.95	99.34	20.12	106.04
4	5000	50	5000	50	14.52	6.52	3.96	69.01	97.21	10.24	0.37
5	50	50	50	50	7.51	4.63	1.36	100	100	0.12	0.25
6	5000	5000	5000	5000	14.81	7.43	4.52	56.57	14.63	6.12	1.7
7	5000	5000	5000	50	9.21	7.11	4.19	65.91	23.34	7.21	0.06
8	50	5000	5000	50	11.78	6.65	4.21	98.63	29.92	12.31	1.03
9	5000	5000	50	50	8.31	6.12	3.36	64.32	18.98	0	1.83
10	5000	50	50	50	8.73	5.32	2.24	78.12	100	0.14	0.17
11	50	50	5000	50	8.12	5.21	3.96	99.32	98.23	10.11	0
12	50	5000	5000	5000	12.93	7.12	4.23	98.63	15.90	1.78	109.37
13	50	50	5000	5000	9.81	6.03	3.19	98.38	98.8	12.76	22.33
14	5000	50	50	5000	10.56	6.91	3.23	74.21	98.63	0	10.32
15	5000	50	50	50	8.41	5.23	2.32	79.97	98.8	0	0.07
16	50	5000	50	50	7.25	5.93	2.43	98.36	41.98	0	0.04
17	5000	5000	50	50	8.63	6.41	2.99	61.36	18.61	0	0.56
18	5000	5000	5000	5000	15.21	7.12	4.83	57.45	12.95	5.65	25.08
19	5000	5000	5000	50	9.21	6.79	4.06	67.95	21.94	6.31	0.13
20	5000	5000	50	5000	10.23	6.43	4.21	69.12	27.90	0.16	12.32
21	50	50	5000	5000	9.73	5.11	3.24	99.28	98.52	14.85	3.69
22	50	5000	5000	50	12.01	7.12	3.99	100	27.93	11.16	0.19
23	5000	50	50	5000	11.36	6.63	3.12	72.92	96.34	0	14.94
24	50	5000	50	50	9.52	5.72	2.63	100	43.32	0	0.3
25	50	50	5000	50	9.52	5.02	2.92	99.32	98.6	9.01	0.17
26	50	50	50	5000	7.52	4.11	1.99	97.9	94.36	5.46	0.53
27	5000	5000	50	5000	9.73	6.36	4.11	69.36	29.91	0.19	11.92
28	50	50	50	5000	7.43	4.03	1.79	99.7	99.1	0.61	40
29	50	5000	50	5000	14.15	7.11	2.74	99.23	23.94	0	29.1
30	5000	50	5000	50	15.36	6.98	3.32	68.5	98.48	14.64	0.48
31	50	5000	50	5000	14.31	6.72	2.96	99.04	21.94	7.27	2.35
32	5000	50	5000	5000	14.23	7.73	3.12	59.01	97.69	18.84	109.3

MDA – nM mg^{-1} protein; Catalase – U mg^{-1} protein; Proline – nM mg^{-1} protein.