PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

HORMESIS EFFECT OF HERBICIDES SUBDOSES ON SUBMERGED MACROPHYTES IN MICROASSAY CONDITIONS

Efeito Hormese de Subdoses de Herbicidas em Macrófitas Submersas em Condição de Microensaio

PERES, L.R.S.[1]
DELLA VECHIA, J.F.[1]
CRUZ, C.[2]*

ABSTRACT - The goal of the study was to evaluate the effect of 2.4-D and clomazone doses on the growth of the submerged macrophytes *Egeria densa* and *E. najas*, in microassay conditions. Therefore, tests were conducted in a bioassay room at the temperature of 27.0 ± 2.0 °C, photoperiod of 24 light hours and illumination of 500 lux. The apical fragments (shoot tips) of the macrophytes with 5.0 cm of length were transferred to test tubes with a 100 mL capacity, containing 70 mL of water. The tested concentrations were: 0.1; 1.0; 3.5; 11.2; 36.5; and 118.0 mg L^{-1} and a control sample with seven replications. In the test with 2.4-D on *E. densa*, in the control sample treatment and the 0.1 mg L^{-1} treatment there was shorter length: at 1.0; 3.5; 11.2; 36.5 and 118.0 mg L^{-1} there was a relative increase of 90.6; 96.3; 91.6; 86.5 and 58.8%, demonstrating growth stimulation. *E. najas* behavior was similar to that of *E. densa*. In the test with clomazone for *E. densa*, the greatest length occurred in the control sample treatment. At the concentrations of 0.1; 1.0; 3.5; 11.2; 36.5 and 118.0 mg L^{-1}, there was relative growth of -25.8; -26.4; -31.7; -28.4; -37.7 and -45.0% respectively, showing herbicidal effect on the plants. *E. najas* behavior was similar, with lower growth at 11.2, 36.5 and 118.0 mg L^{-1}. Sub-doses of the herbicide 2.4-D cause growth stimulation (Hormesis effect) in *E. densa* and *E. najas,* while clomazone causes herbicidal effect.

Keywords: growth stimulation, aquatic plants, environmental dynamics, pesticides.

RESUMO - O objetivo deste estudo foi avaliar o efeito de subdoses dos herbicidas 2,4-D e clomazone no crescimento das macrófitas submersas ***Egeria densa*** *e* ***E. najas****, em condição de microensaio. Para isso, os ensaios foram conduzidos em sala de bioensaio com temperatura de 27,0 ± 2,0 °C, fotoperíodo de 24 horas de luz e iluminação de 500 lux. Os fragmentos apicais (ponteiros) das macrófitas com 5,0 cm de comprimento foram transferidos para tubos de ensaio com capacidade de 100 mL, contendo 70 mL de água. As concentrações testadas foram: 0,1; 1,0; 3,5; 11,2; 36,5; e 118,0 mg L^{-1}, e um controle com sete repetições. No ensaio com 2,4-D, para* ***E. densa****, no controle e em 0,1 mg L^{-1} ocorreu menor comprimento; em 1,0, 3,5, 11,2, 36,5 e 118,0 mg L^{-1} ocorreu crescimento relativo de 90,6, 96,3, 91,6, 86,5 e 58,8%, demonstrando estímulo do crescimento. Para* ***E. najas****, o comportamento foi similar ao de* ***E. densa****. No ensaio com clomazone, para* ***E. densa****, o maior comprimento ocorreu no controle. Nas concentrações de 0,1; 1,0; 3,5; 11,2; 36,5; e 118,0 mg L^{-1} ocorreu crescimento relativo de -25,8; -26,4; -31,7; -28,4; -37,7; e -45,0%, respectivamente, demonstrando efeito herbicida para a planta. Quanto a* ***E. najas****, o comportamento foi similar, com os menores crescimento em 11, 2, 36,5 e 118,0 mg L^{-1}. O herbicida 2,4-D, em subdoses, causa estímulo no crescimento (efeito hormese) de* ***E. densa*** *e* ***E. najas****, ao passo que o clomazone provoca efeito herbicida.*

Palavras-chave: estímulo de crescimento, plantas aquáticas, dinâmica ambiental, agrotóxicos.

*** Corresponding author:**
<claudineicruz@gmail.com>

Received: June 28, 2016
Approved: August 15, 2016

Planta Daninha 2017; v35:e017165857

[1] Universidade Estadual Paulista, São Paulo-SP; [2] Centro Universitário da Fundação Educacional de Barretos, São Paulo-SP, Brasil.

Doi: 10.1590/S0100-83582017350100076

INTRODUCTION

Macrophytes are responsible for the biodiversity and spatial heterogeneity of water ecosystems, with ecological importance for the creation of habitats for organisms, nutrient retention, protection of water bodies banks, as well as contributing to the global decline of the carbon stock (Olson and Doherty, 2014). However, some factors may contribute to the growth of mono-species colonizations, such as industrial and domestic waste (Maximiano et al., 2005), fertilizers (von Sperling, 2011), the application technology used and toxicity of the formulation (Primel et al., 2005), erosive processes and transportation of particulate matter coming from agriculture (Yadav et al., 2015).

Studies about the environmental dynamics of herbicides in water environments and the effects over macrophytes have been described with terbutylazine in *Callitrine carpa*, *Ceratophyllum demersum*, *Elodea canadense*, *Potamogeton crispus* and *Miriophyllum spicatum* (Cedergreen et al., 2004); with 2.4-D on *Lemna trisulca*, *Elodea nuttallii*, *C. demersum* and *Potamogeton lucens* (Belgers et al., 2007); with 50% atrazine + 35% isoproturon + 15% alachlor on *Azolla filiculoides*, *C. demersum*, *Elodea canadensis*, *Lemna minor*, *M. spicatum* and *Vallisneria spiralis* (Coutris et al., 2011); and with atrazine on *E. canadensis* (Brain et al., 2012). Thus, among the possible anthropic activities that have an impact on water environments, the use of pesticides, especially herbicides (Morh et al., 2007; Yadav et al., 2015), may be a factor in the colonization or not by macrophytes.

Herbicides can have an impact on the macrophyte community, since they can affect directly the water environments, when used to control macrophytes (Cruz et al., 2015) and algae (Garlich et al., 2016), or also indirectly, when coming from agriculture (Yadav et al., 2015). Among the effects involving the presence of herbicide sub-doses on plants, there is hormesis, which consists in the dose-response relation, at low concentrations, in opposition to what occurs at high doses (Calabrese and Blain, 2002, 2011), which indicates a possible adaptation to the stress condition (Birringer, 2011). Thus, at low doses stimulation occurs, whereas high doses inhibit plant growth.

Among herbicides, 2.4-D (2.4-dichlorophenoxyacetic acid) is a growth regulator that has a similar effect to the ones of the auxin hormone, and clomazone [2-[methyl(2-chlorophenyl)]-4,4-dimethyl-3-isoxazolidinone] is widely used to control weeds from different annual cultures. Thus, one of the problems created by the intensification of agricultural productive systems is the indirect release of pesticide sub-doses into water bodies; this may contribute to a change in the population patterns of macrophytes, interfering directly in the development or suppression of their communities, which may contribute to alterations in the quality standards of a determined environment. The goal of this study was to determine the effect on the growth of the submerged macrophytes *Egeria densa* and *E. najas* exposed to sub-doses of 2.4-D and clomazone, under microassay conditions.

MATERIAL AND METHODS

The tested products were 2.4-D (dichlorophenoxyacetic acid - CAS 94-75-7) with 806.0 g L^{-1} of active ingredient, in the DMA® BR formulation, and clomazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone - CAS 81777-89-1) with 360 g L^{1}, in the Gamit® 360 CS formulation.

In order to conduct the tests, macrophytes were cultivated (*E.densa* e *E.najas*) in 200 l boxes with bottom sediment composed by Latosol, sand and organic compost (1:1:1; v/v). After their growth in occupying the box, the best shoot tips (with a healthy aspect) were selected in order to run the tests (Henares et al., 2011).

Tests were conducted in a bioassay room with a temperature of 27.0 ± 2.0 °C, a photoperiod of 24 light hours and lighting of 500 lux. To do so, apical fragments (shoot tips) from the macrophytes, 5.0 cm long, were collected and transferred to test tubes with 100 mL capacity, containing 70 mL of water (pH 7.0, electrical conductivity at 170.0 µS cm^{-1} and dissolved oxygen > 4.0 mg L^{-1} - previously measured with a YSI Plus multi-parameter probe), where they remained for 24 to acclimate. After that, the following concentrations were tested: 0.1, 1.0, 3.5, and 11.2, 36.5 and 118.0 mg L^{-1} of each herbicide and a control sample (control sample without herbicide addition, with seven replications per concentration, with two tests per each macrophyte.

The exposure period of macrophytes to the herbicides was seven days. At the end of this period, the shoot tips were evaluated as for length (cm) and fresh biomass (g). Visual evaluations about phytotoxicity (chlorosis and necrosis) were performed three, five and seven days after the application of the products. At the end of the experimental period, plants were removed from the containers and the final growth length (cm) was obtained with the help of a digital pachymeter. The obtained data were submitted to analysis of variance (ANOVA) and the averages were compared by Tukey's test at 5%.

RESULTS AND DISCUSSION

In tests with 2.4-D for *E. densa*, in the control sample and at 0.1 mg L^{-1}, there were shorter shoot tips, differing significantly from the other treatments. At 1.0, 11.2 mg L^{-1} there was relative growth of 90.6 and 91.6% in the first test, and 51.2 and 64.8% in the second test. Also at 36.5 and 118.0 mg L^{-1} there was stimulation for the relative growth in relation to control, without having significant differences (Table 1). The 3.5 mg L^{-1} concentration stood out, because it had the highest relative growth (96.3 and 77.75%), but there were no significant differences among the other treatments (Figure 1). As for the final weight (g), there were no significant differences among the treatments, with a variation between 0.8 and 1.0 grams.

Table 1 - Average ± deviation of the final length (cm) of macrophytes *E. densa* and *E. najas* shoot tips, exposed to 2.4-D

	E. densa (mg L^{-1})						
	0.0	0.1	1.0	3.5	11.2	36.5	118.0
1*Average ± STD**	7.6±2.0b	7.6±1.5b	14.5±1.6a	15.0±2.4a	14.6±2.7a	14.2±1.9a	12.1±2.7a
% growth	100.0	100.5	190.6	196.3	191.6	186.5	158.8
2*Average ± STD**	11.5±20bc	10.9±2.3c	17.5±5.3ab	20.5±4.3a	19.0±5.4a	17.0±2.3abc	15.3±1.7abc
% growth	100.0	94.4	151.2	177.7	164.8	146.9	132.7
	E. najas (mg L^{-1})						
1*Average ± STD**	10.0±1.3b	10.2±1.2b	17.6±2.5a	11.6±4.1b	12.7±2.2b	13.3±2.4b	11.0±1.2b
% growth	100.0	102.1	175.0	115.5	126.8	132.5	109.1
2*Average ± STD**	12.7±1.8d	16.5±3.0cd	22.6±2.7a	21.8± 3.5ab	19.4±3.6abc	18.2±2.9abc	17.7±2.0bc
% growth	100.0	129.6	177.0	170.7	151.9	142.4	138.5

1, 2 = number of experiments; * Lines followed by lowercase letters compare between their averages; ** STD = standard deviation of the average.

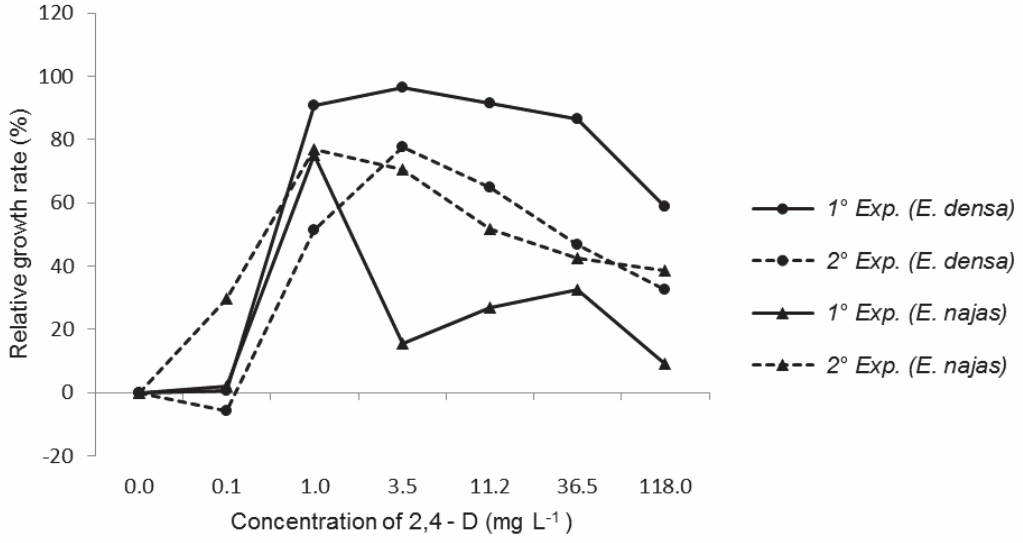

Figure 1 - Relative growth rate (%) of the macrophytes *E. densa* and *E. najas* exposed to 2.4-D.

At 1.0 to 11.0 mg L^{-1} of 2.4-D there was growth stimulation of the shoot tip of *E. densa* (Figure 1), differing from 510.0, 1,000 and 2,000 µg L^{-1} of atrazine with *E. canadensis*, with final growth similar to the control sample as for luminosity of 600 lux (Brain et al., 2012), and for terbutylazine at the concentrations of 1.0 to 1,000.0 µg L^{-1}, with growth reduction for *Lemna minor*, *L. trisulca*, *Spirodela polyrrhiza*, *Callitricheplaty carpa*, *Myriophyllum spicatum*, *Elodea canadensis*, *Potamogeton crispus*, *Ceratophylum submersum* and *C. demersum* (Cedergreen et al., 2004). However, the response of this study was similar to the one described for 2.4-D at the concentration of 30.0 µg L^{-1} for *Elodea nutalli*, *Ranunculus circinatus* and *R. aquaticus* and at 30.0 and 100.0 µg L^{-1} for *Ceratophylum demersum* and *Potamogeton crispus* (Belgers et al., 2007), with stimulation to the growth of macrophytes.

In the test with 2.4-D for *E. najas*, at 1.0 mg L^{-1} there was a relative growth of 75.0 and 77%, standing out for the fact that growth was higher in relation to the others; it was significantly different from the other concentrations (Table 1). The lowest length occurred in the control sample and in the 0.1 mg L^{-1} treatment, but there were no significant differences between the treatments. At the concentrations of 3.5, 11.2, 36.5 and 118.0 mg L^{-1}, values remained around 20 to 50% (Table 1). The herbicide 2.4-D, applied in sub-doses, causes growth stimulation of *E. densa*. In this plant's case, there was an increase in the relative growth rate in relation to the control sample. As for *E. najas*, the highest relative growth rate (75 and 77% in the 1st and 2nd test, respectively) occurred with the exposure to 1.0 mg L^{-1} (Figure 1). As for the final fresh weight (g), there were no significant differences between the treatments in the two control samples, with final average varying between 0.8 and 1.0 g.

The presence of herbicides in surface waters may cause a series of environmental problems to the non-target water vegetation, especially at low doses (Grossmann and Kwiatowski, 2000). The herbicides bensulfuron-methyl (0.0005 and 0.001 mg L^{-1}) and atrazine (0.0005 and 0.001 mg L^{-1}) also caused an increase in the relative growth rate for the submersed macrophyte *C. demersum* from 60.14 and 47.11% in relation to the control sample (Pan et al., 2009), similar to the one of 0.1 mg L^{-1} of 2.4-D for *E. densa* and *E. najas*.

In tests with clomazone, for *E. densa*, the lowest length occurred in the treatment with 118.0 mg L^{-1}, which was significantly different from the other concentrations (Table 2). In the control sample and the 0.1 mg L^{-1} treatment there was greater growth; this differed significantly from the other concentrations (Table 2). At 1.0, 3.5 and 11.2 mg L^{-1} there were no significant differences, with intermediate growth of the shoot tips in relation to the other treatments (Table 2). At 3.5, 11.2 and 36.5 mg L^{-1} there were no significant differences, with similar values. The relative growth rate was negative in relation to the control sample; it was -14.5, -19.8, -18.3, -25.9 and -36.6% (Figure 2). As for fresh weight, there were no significant differences among the evaluated treatments, with values around 0.7 to 0.5 g in all of them. The herbicide effect of clomazone occurred starting from 0.1 mg L^{-1}, similarly to diquat at the concentrations of 0.5 and

Table 2 - Average ± deviation of the final length (cm) of macrophytes *E. densa* and *E. najas* shoot tips, exposed to clomazone (mg L^{-1})

	E. densa (mg L^{-1})						
	0.0	0.1	1.0	3.5	11.2	36.5	118.0
1*Average ± STD**	9.3±0.9a	8.5±0.9ab	8.0±0.8bc	7.5±0.5cd	7.6±1.2bcd	6.9±1.0d	5.9±0.5e
% growth	100.0	90.8	85.5	88.2	81.6	74.0	63.3
2*Average ± STD**	10.7±2.0a	8.0±1.2bc	7.9±0.8bc	7.3±0.7bc	7.7±1.4bc	6.7±1.0bc	5.9±0.6c
% growth	100.0	74.1	73.5	68.2	71.5	62.2	54.9
	E. najas (mg L^{-1})						
1*Average ± STD**	9.2±1.2abc	11.6±2.6a	11.7±3.3a	11.2± 2.9ab	8.5±1.0abc	7.7±1.9bc	7.0±0.7c
% growth	100.0	126.3	127.1	122.4	93.0	84.5	76.7
2*Average ± STD**	8.5±0.8a	8.5±0.9a	8.4±0.8a	7.7±0.7a	7.7±1.4a	7.7±1.9a	5.7±0.2b
% growth	100.0	100.0	98.3	90.8	89.1	90.8	66.6

1, 2 = number of experiments; * Lines followed by lowercase letters compare between their averages; ** STD = standard deviation of the average.

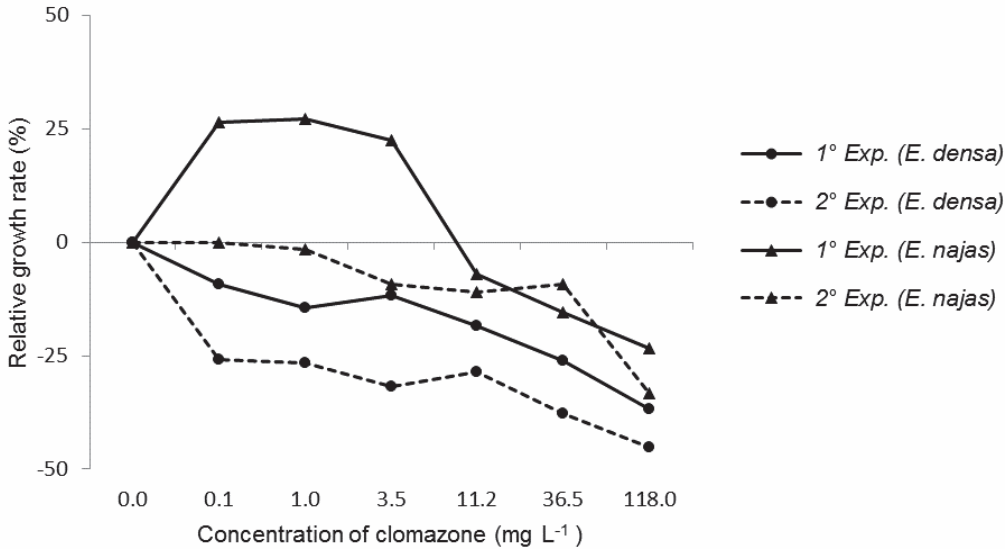

Figure 2 - Relative growth rate (%) of the macrophytes *E. densa* and *E. najas* exposed to clomazone.

1.0 mg L^{-1} for *E. densa* and *E. najas* (Martins et al., 2007) and 0.4 to 1.6 mg L^{-1} for *Hydrilla verticillata*, with an 80% reduction of the biomass (Henares et al., 2011).

In the experiment with clomazone, for *E. najas* the lowest growths were at 11.2, 36.5 and 118.0 mg L^{-1}, with no significant differences in relation to the control sample (Table 2); and at 0.1 and 1.0 mg L^{-1}, it was similar to the others, except at 3.5 and 11.2 mg L^{-1} (Table 2). At 0.1, 1.0, 3.5, 11.2, 36.5 and 118.0 mg L^{-1} the relative growth of plants was 26.3, 27.1, 22.4, -6.9, -15.5 and -23.2% respectively (Figure 2). It is important to highlight that starting from the treatment with 11.2 mg L^{-1}, there was herbicide effect in controlling the macrophyte. As for the fresh weight (g), there were no significant differences among the treatments.

The presence of clomazone sub-doses causes herbicide effect on both macrophytes. Sub-doses of 1.0, 3.5 and 11.2 mg L^{-1} of 2.4-D caused growth stimulation for *E. densa* and *E. najas*, indicating hormesis effect of this herbicide on submersed macrophytes, creating a new problem to be evaluated, because of the presence of waste of sub-doses of these products in the water environment.

REFERENCES

Brain R.A. et al. Influence of light intensity on the toxicity of atrazine to the submerged freshwater aquatic macrophyte *Elodea canadenses*. **Ecotoxicol Environ Safety**. 2012;79:55-61.

Belgers J.D. et al. Effects of the herbicide 2.4-D on the growth of nine aquatic macrophytes. **Aquatic Bot.** 2007;86:260-8.

Birringer M. Hormetics: dietary triggers of an adaptive stress response. **Pharm Res.** 2011;28:2680-94.

Calabrese E.J., Baldwin L.A. Defining hormesis. **Human Exper Toxicol.** 2002;21:91-7.

Calabrese E.J., Baldwin L.A. The hormesis database: The occurence of hermetic dose responses in the toxicological literature. **Regul Toxicol Pharm.** 2011;61:73-81.

Coutris C. et al. A. Can we predict community-wide effects of herbicides from toxicity tests on macrophyte species. **Aquatic Toxicol.** 2011;101:49-56.

Cedergreen N. et al. Species-specific sensitivity of aquatic macrophytes towards two herbicide. **Ecotoxicol Environ Safety**. 2004;58:314-23.

Cruz C. et al. Imazapyr herbicide efficacy on floating macrophyte control and ecotoxicology for non - target organisms. **Planta Daninha**. 2015;33:103-8.

Garlich N. et al. Diquat associated with copper sources for algae control: Efficacy and ecotoxicology. **J Environ Sci Health Part B.** 2016;51:215-21.

Henares M.N.P. et al. Eficácia do diquat no controle de *Hydrilla verticillata, Egeria densa* e *Egeria najas* e toxicidade aguda para o Guaru (*Phallocerus caudimaculatus*), em condições de laboratório. **Planta Daninha**. 2011;29:279-85.

Grossmann K., Kwiatkowski J. The mechanism of quinclorac selectivity in grasses. **Pest Biochem Physiol.** 2000;66:83-91.

Martins D. et al. Sensibilidade de diferentes acessos de *Egeria najas* e *Egeria densa* aos herbicidas diquat e fluridone. **Planta Daninha**. 2007;25:351-8.

Maximiano A.A. et al. Utilização de drogas veterinárias, agrotóxicos e afins em ambientes hídricos: demandas, regulamentação e considerações 16 sobre riscos à saúde humana e ambiental. **Ci Saúde Coletiva**. 2005;10:483-91.

Mohr S. et al. Effects of the herbicide metazachlor on macrophytes and ecosystem function in freshwater pond and stream mesocosms. **Aquatic Toxicol.** 2007;82:73-84.

Olson E.R., Doherty J.D. Macrophyte diversity–abundance relationship with respect to invasive and native dominants. **Aquatic Bot.** 2014;119:111-9.

Pan H. et al. Phytotoxicity of four herbicides on *Ceratophyllum demersum, Vallisneria natans* and *Elodea nuttalli*. **J Environ Sci.** 2009;21:307-12.

Primel E.G. et al. Poluição das águas por herbicidas utilizados no cultivo de arroz irrigado na região central do estado do Rio Grande do sul, Brasil: Predição teórica e monitoramento. **Quím Nova**. 2005;28:605-9.

von Sperling M. **Introdução á qualidade das águas e ao tratamento de esgotos**. Belo Horizonte: DESA/UFMG, 2011. 452p.

Yadav I.C. et al. Current status of persistent organic pesticides residues in air, water, and soil and their possible effect on neighboring countries: A comprehensive review of India. **Sci Total Environ.** 2015;511:127-37.