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Bioremediation potential of *Lentinus squarrosulus* (Mont.) Singer. A Nigerian white rot fungus to biodegrade brewery wastewater

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ABSTRACT: The natural ability of *Lentinus squarrosulus* (Mont.) Singer to utilize inorganic metals as a bioremediation agent in a Nigerian brewery waste-water (WW) was investigated. The results obtained showed that the levels of inorganic metals assessed, Na, Ca, Mg, Zn, Co, Cu, Cd, P, and Pb except K, in the brewery WW was significantly reduced when inoculated with *L. squarrosulus*, after incubation periods of 5, 10, 15, 20 and 25 days. It was also observed that levels of the minerals (except Na and K) in the pure strain of *L. squarrosulus* were significantly reduced in the mycelia but Na was significantly increased while that of K was constant. There was an increase in the mycelia biomass from incubation period of day 5 (0.86 mg/g ±0.309) to a peak at day 10 (0.90mg/g ±0.316) with a fall at day15 (0.85mg/g ±0.397), and no significant mycelia growth was observed at day 20 (0.15mg/g ±0.000) and day 25 (0.001 ±0.000) respectively. This study revealed that *L. squarrosulus* could bioaccumulate and tolerate non- essential metals such as Pb and Cd. The fungus *L. squarrosulus* (Mont.) Singer, an indigenous Nigerian mushroom used in this study, was observed to posses the potential for biosorption of inorganic metals in brewery wastewater, and therefore, it could help in bioremediation of non-essential metals and metal toxicity for a safe environment and improved health

Key words: *Lentinus squarrosulus,* bioremediation, brewery wastewater, mycelia, inorganic metals

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INTRODUCTION

One of the major environmental challenges facing the world today is the contamination of the environment by toxic chemicals such as inorganic metals from industrial wastes (Abioye, 2011). Wastewater discharged from industries into water bodies are known to cause deleterious effects on fauna and flora of lakes and streams, and consequently, exert severe effects on the ecosystem and people (WHO, 1984; Mahavi, 2005; Sayari *et al*., 2005 and Damini *et al*., 2013). Conventional processes for removal of inorganic metals from industrial wastewaters include chemical precipitation, oxidation-reduction, filtration and electrochemical techniques (Damini *et al*., 2013). However, these processes cannot be environmentally friendly, fully efficient and present very high cost (Damini *et al*., 2013). Biological processes such as biosorption or bioaccumulation are advocated in recent years because of the main drawbacks in chemical treatment processes (Hussein *et al*., 2004; Damini *et al*., 2013 and Akpor *et al*., 2014). The main advantages of biosorption over conventional treatment methods include low cost, high efficiency, minimization of chemical and biological sludge, regeneration of biosorbent and possibility of metal recovery (Kratohvil and Volesky, 1998; Yalinkay *et al*., 2002; Hussein *et al*., 2004 and Preetha and Viruthagiri, 2005). Microbial remediation to

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reduce the concentration or toxicity of pollutants is an efficient and economical approach that has been successful in laboratory studies (Akpor *et al*., 2014). *Lentinus squarrosulus* (M) Singer is a highly priced edible indigenous Nigerian mushroom species (Nwanze *et al*., 2008; Adejoye *et al*., 2012). It belongs to the family of polyporaceae or lentinaceae (Fasidi and Kadiri 1995; Adejoye *et al*., 2009). Adejoye *et al*., 2012 recently reported the use of *L. squarrosulus* for human benefits and also its ability to produce exopolysaccharide important in medicine. The objective of this study is to assess the potential of *Lentinus squarrosulus* (Mont.) Singer, an indigenous Nigerian mushroom to utilize the inorganic metals composition of a Nigerian brewery wastewater.

MATERIALS AND METHODS

Waste water samples and preparation

One liter effluent sample was collected from Consolidated Brewery Nigeria Limited along Ijebu Ode-Epe express road, Ijebu-Ode, Ogun State, Nigeria, in a sterile plastic container, transported immediately to the laboratory and stored at 4 $\pm 2^0$C for use to avoid further degradation.

Microorganism sample and culture conditions

The culture of *Lentinus squarrosulus* used was obtained from the laboratory of Biological Sciences Department, Tai Solarin University of Education. Ijagun, Ijebu-Ode, Ogun State, Nigeria. The culture was maintained on 4% malt extract agar medium at 22^{o}C.

Experimental setup

The effluent sample was apportioned into 18 aliquots of 30 ml and sterilized at 121^{0}C for 15 min. Thereafter, the aliquot samples were maintained at ambient temperature (28 $\pm$ 2^{0}C). Fifteen of the parts of these portions were inoculated with 1.00 gm of *L. squarrosulus* and incubated for 5, 10, 15, 20 and 25 days. Each treatment was prepared in triplicate. Three controls were set up thus: sterilized effluent, pure strain of *L. squarrosulus* (positive control) and double distilled water (ddH_2O) only (negative control).

Determination of mycelial biomass

The fungus mycelia in the test samples were determined by measuring the dry weight of the biomass yield. The mycelia from each sample was filtered through a mesh of 30mm pore size and washed with distilled water. The collected mycelial was filtered through a pre-weighted Whatman filter paper No. 1, after which freeze drying and weighing was repeated until a constant dry weight was obtained. The filtrate was collected and refrigerated at 4^{o}C for determination of inorganic metal content.

Inorganic metal analysis

The mycelial mass of the fungus was analyzed for inorganic elements contents at the end of each incubation period. The filtrate after each harvest was also analyzed to determine the level of metallic element present. The three controls were also analyzed for inorganic elements contents. Levels of Na, Ca, K and Mg in each sample were determined using flame photometry (AOAC, 1998); Cu, Pb, Cd, Zn, and Co by atomic absorption spectrophotometry (AOAC, 1998); and P by colorimetric method (Murphy and Riley, 1962).

Statistical analysis

The data obtained from this research were analysed using Statistics Analysis System (SAS 9.2) for precision measures, Analysis of Variance (ANOVA); Duncan Multiple Range Test using general linear model option, and correlation analysis was based on Pearson coefficient method.

RESULTS AND DISCUSSION

The obtained results showed that both the essential and non-essential metals were present in the brewery waste water, pure strain of *L. squarrosulus*, mycelia and distilled water at varying levels (Table 1). The present results support that industrial processes generate large amounts of liquid effluent containing metals such as copper, cadmium, chromium and zinc (Veglio *et al*., 2003). All the assessed samples contained Na at high level followed by K and Ca while the level of P was the least, except in the negative control (Table 1). The decreasing order of the abundance of the minerals across the samples was as follows Na > K > Ca > Zn > Mg > Cu > Co > Pb > Cd > P, with a little variation after Cu, among the samples ($p<0.05$). The observed results might indicate the abundance of the essential minerals (Na, K and Ca, Zn, Mg, Cu and Co) among the assessed metals, the order of their requirement for metabolic processes and their availability in nature. The analysis of variance results showed a very highly significant variation of these various inorganic metals among the different sample materials. The highest amounts of each of Na, K, Ca, Mg, Zn, Cu, Co, Pb, and Cd were obtained in mycelia biomass, filtrate, effluent and the pure strain respectively. The least quantities of these elements were obtained in the pure strain, mycelia biomass and filtrate. From these results, it was observed pure strain of *L. squarrosulus* had the highest levels in six out of the ten assessed metals (Mg, Cu, Zn, Pb and Cd). These results, therefore, indicated natural bioaccumulation of inorganic metals by *L. squarrosulus* and their importance in its metabolic life processes which is in agreement with the findings of Chadrasekaran and Rajkannan (2003), and also, perhaps its ability to resist and survive their toxicity during bioaccumulation. Duncan multiple range tests (DMRT) based on general linear model showed a significant difference ($P<0.05$) among the various samples and placed the samples in different groups as depicted in Table 2.

Duncan multiple range test categorized the samples into different classes based on the significant mean difference (Table 2). When the results obtained for the different filtrates were compared, it was observed that the levels of both the essential (except K) and non-essential metals assesses in the effluent were significantly reduced ($P<0.05$) in the filtrate (Fig. 1a), indicating that metal ions uptake by *L. squarrosulus* were being utilized for metabolic life process to obtain mycelia biomass. The higher level of K found in the filtrate could not be accounted for because of the lower level obtained (0.39mg/g) for both the effluent and the pure strain, which was overshoot in the filtrate (0.71 mg/g). The results also revealed that the levels of the assessed metals (except Na and K) in the pure strain of *L. squarrosulus* were significantly reduced ($P<0.05$) in the mycelia, Na was significantly increased in the mycelia but that of K was statistically non-significant ($P<0.05$) and were almost the same amount (Fig. 1b). The reduced values of the metals in the *L. squarrosulus* could be a result of their use up for the production of the mycelia biomass while Na and K, as macro-essential metals, are required in large quantity, and were bioaccumulated for further use in maintaining the mycelia metabolic life processes, but K was required in a less reduced amount than Na. The report of Banerjee et al., (2007) supported the present findings that wastewater from industries contained trace metal which are directly or indirectly involved in all aspects of microbial growth. Gnanadoss and Jebapriya, 2013 and Bayramoglu *et al*., 2003 have also reported that white rot fungi have abilities to accumulate metals from industrial wastewater The *L. squarrosulus* used in this study was therefore, able to bioabsorp the minerals in the effluent and utilized them for its metabolic processes and further bioaccumulated Na for

Table 1: Descriptive statistics of assessed minerals in the effluent, *Lentinus squarrosulus*, cultured filtrate, and harvested mycelia

Samples	Inorganic metals	Mean (mg/g)	Standard deviation	Standard error(±)	Coefficient of variation	Range	Minimum	Maximum
Effluent	Na	1.5000	1.48	1.05	98.99	2.1	0.45	2.55
	K	0.3900	0.0283	0.02	7.2524	0.04	0.37	0.41
	Ca	0.0100	0.0002	0.02	1.6288	0.0002	0.0099	0.0101
	P	0.0003	0.0001	0.01	22.6274	0.0001	0.0002	0.0003
	Mg	0.0053	0.00	0	0.6677	0	0.0053	0.0053
	Cu	0.0042	0	0	1.0174	0.0001	0.0041	0.0042
	Zn	0.0053	0.00	0	0.6615	0	0.0053	0.0054
	Co	0.0033	0.0001	0.01	2.3823	0.0001	0.0032	0.0033
	Pb	0.0031	0.00	0	0.6767	0	0.0031	0.0032
	Cd	0.0019	0.0001	0.01	3.009	0.0001	0.0018	0.0019
	Na	0.5800	0.0141	0.01	2.4383	0.02	0.57	0.59
	K	0.3900	0.0141	0.01	3.6262	0.02	0.38	0.4
	Ca	0.0085	0	0	0.3339	0	0.0085	0.0085
	P	0.0003	0	0	4.2855	0	0.0003	0.0003
	Mg	0.0062	0	0	0.6899	0.0001	0.0061	0.0062
Pure	Cu	0.0050	0.0001	0.0001	1.5418	0.0001	0.005	0.0051
strain	Zn	0.0062	0	0	0.3435	0	0.0062	0.0062
	Co	0.0031	0	0	0.2248	0	0.0031	0.0032
	Pb	0.0036	0.0001	0	1.7507	0.0001	0.0036	0.0037
	Cd	0.0020	0	0	1.0741	0	0.002	0.002
	Na	1.6653	0.2015	0.0475	12.0995	0.75	1.35	2.1
	K	0.4156	0.1026	0.0242	24.696	0.42	0.17	0.59
	Ca	0.0032	0.0005	0.0001	14.4768	0.0017	0.0025	0.0042
	P	0.0001	0	0	69.8137	0.0002	0	0.0002
Mycelia	Mg	0.0017	0.0002	0	11.0972	0.0007	0.0013	0.002
	Cu	0.0010	0.0003	0.0001	25.5088	0.001	0.0005	0.0015
	Zn	0.0020	0.0002	0	8.3346	0.0005	0.0017	0.0022
	Co	0.0007	0.0002	0	28.3008	0.0006	0.0003	0.0009
	Pb	0.0002	0.0001	0	27.0952	0.0002	0.0001	0.0003
	Cd	0.0001	0.0001	0	36.4964	0.0002	0	0.0002
	Na	0.6567	0.148	0.027	22.5328	0.6	0.35	0.95
	K	0.7080	0.1751	0.032	24.7364	0.79	0.16	0.95
	Ca	0.0079	0.0009	0.0002	11.8564	0.0034	0.0066	0.0099
	P	0.0001	0	0	25.056	0.0002	0.0001	0.0002
	Mg	0.0030	0.0006	0.0001	18.8517	0.0027	0.0015	0.0042
	Cu	0.0029	0.0007	0.0001	23.1038	0.0021	0.0019	0.004
Filtrate	Zn	0.003	0.0005	0.0001	14.0796	0.0017	0.003	0.0047
	Co	0.0024	0.0006	0.0001	25.1499	0.0018	0.0014	0.0033
	Pb	0.0022	0.0002	0	9.7577	0.0008	0.0019	0.0027
	Cd	0.0016	0.0002	0	10.9205	0.0007	0.0013	0.002
	Na	1.0250	0.0224	0.0091	2.1815	0.05	1	1.05
	K	0.4917	0.0232	0.0095	4.7117	0.07	0.46	0.53
	Ca	0.0094	0.0003	0.0001	3.1683	0.0006	0.0091	0.0098
	P	0.0003	0	0	6.2012	0	0.0002	0.0003
	Mg	0.0045	0.0001	0	2.1758	0.0003	0.0044	0.0047
Control	Cu	0.0041	0.0001	0	1.8517	0.0002	0.004	0.0041
	Zn	0.0054	0.0000	0	0.8789	0.0001	0.0054	0.0055
	Co	0.0028	0.0001	0	2.5954	0.0002	0.0027	0.0028
	Pb	0.0031	0.0000	0	1.038	0.0001	0.003	0.0031
	Cd	0.0018	0	0	1.326	0.0001	0.0018	0.0019

Table 2: Significant variation of minerals among effluent, *L. squarrosulus*, cultured filtrate, harvested mycelia and incubating periods based on Duncan multiple range test†

Samples	Variation among samples									
	Inorganic metals									
	Na	K	Mg	Ca	P	Pb	Zn	Cu	Co	Cd
Control	B	B	C	BA	B	B	B	B	BA	A
Effluent	A	B	B	A	B	B	B	B	A	A
filtrate	C	A	D	C	C	C	C	C	B	B
Pure strain	C	B	A	BC	A	A	A	A	BA	A
Mycelia	A	B	E	D	D	D	D	D	C	C
Day	**Variation among Incubating periods**									
0	A	B	A	A	A	A	A	A	A	A
5	A	BA	CD	B	C	B	C	B	B	B
10	A	BA	CD	B	C	B	C	B	B	B
15	A	B	D	B	C	B	C	B	B	B
20	A	A	CB	BA	CB	A	CB	B	B	A
25	A	BA	B	BA	B	A	B	B	B	BA

The variation observed was highly significant at p d" 0.0005,
†Duncan multiple range test grouped the parameters based on separation of means, different letters were assigned to parameters if there are significant difference in their means but parameters with the same letters are not significant different in their means

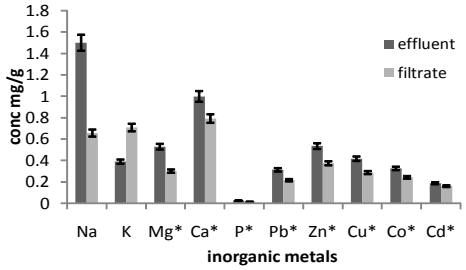

Fig. 1a. Comparison level of metals in brewery effluent and filtrate inocculum

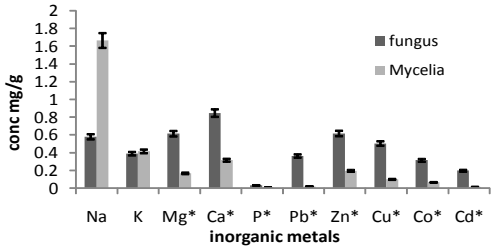

Fig. 1b. Comparison level of metals in pure *L. squarrosulus* and harvested myclia

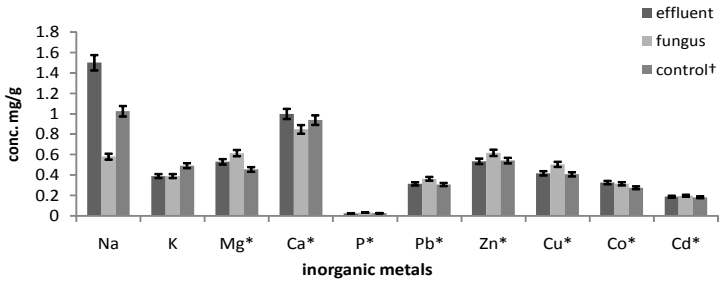

Fig. 1c. Comparison level of metals in brewery effluent, *L. squarosulus* and control

* Multiplied the value by a factor of 100, † Distill water without inoculum

possible cell functions in mycelia growth. This present study further supports the report of Yetis *et al*., (2006) that higher absorption of macro-nutrients by white rot fungi is necessary for metabolic activities and cell building.

The mean concentrations obtained showed that the levels of Na, Mg, Ca, and Co were significantly higher in the effluent than the negative control while the levels of K, Cd, Cu, P, Pb and Zn were not significantly different (Fig 1c). This result indicated the importance of Na, Mg, Ca and Co in brewing industries and as by-products. In addition, Na pellet is a known for buffering brewing waste water, as a treatment to obtain a neutral pH, before discharge, in other to minimize its hazardous effect on aquatic lives. The insignificant levels of K, Cd, Cu, Pb, and Zn in the brewing wastewater when compared with the control could be because of pre-treatment of the waste water before its discharge. It was also observed that the respective amounts of Mg, P, Pb, Zn, and Cu were higher in the pure strain of *L. squarrosulus* than the control while Na and Ca were significantly higher ($P<0.05$) in control than the pure strain but with no significant difference ($P<0.05$) for K, Co and Cd (Fig 1c). This observation indicates that *L. squarrusolus* do bioaccumulate some micro essential metals such as Mg and Zn for normal cell functions; has enough K, Ca and Co that is required for its life processes and could tolerate high levels of Pb and Cd that pass through the free access gate, but requires large amount of Na from external sources. In confirming this, it was observed that the amount of Na was higher in the effluent than the pure strain but

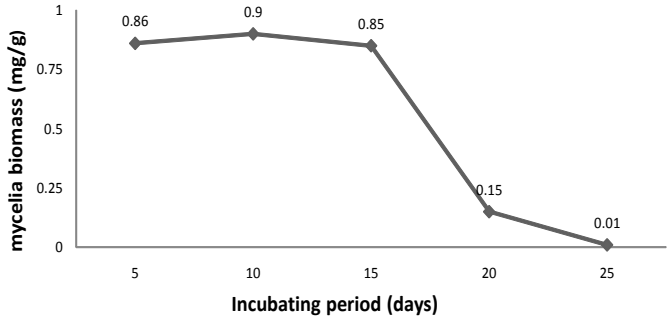

Fig. 2a. Trend in mycelial biomass over incubation period

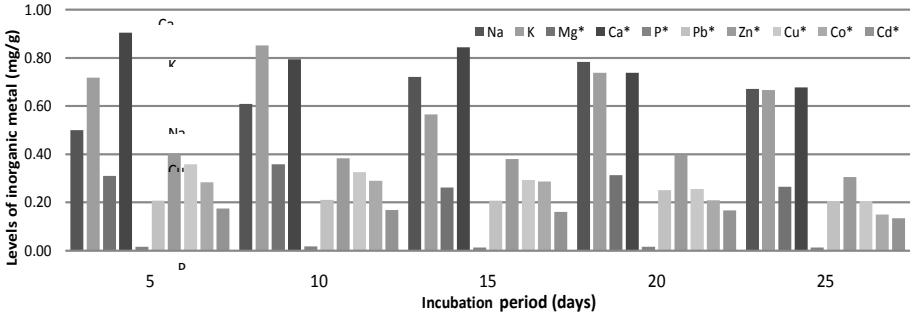

Fig. 2b. Trend in the level of metal over incubation period

bioaccumulated in the mycelia as shown in fig 1c. Therefore, *L. squarrosulus* took up a lot of Na from the effluent and bioaccumulated it in the mycelia for further cell functions. In addition, Na is known to function as an electrolyte in signal transduction in cells. Hence, the importance of Na and its required large quantity in *L. squarrosulus* for proper cell functions is highlighted in the present study.

The amount of harvested mycelia biomass at day 5, 10, 15, 20 and 25 were 0.86 ± 0.309 mg/g; 0.90± 0.316 mg/g; 0.85 ± 0.307 mg/g; 0.15± 0.000 mg/g and 0.01 ±0.000 mg/g respectively. These results showed that nutrients in the wastewater from the examined brewery supported the growth of *L. squarrosulus* at day 5 to 10 days however a significant reduction in mycelial biomass was observed at day 20 and 25 (Fig.2). The present observation agreed with many reported findings that essential metals such as K, Na, Mg, Ca, Mn, Fe, Cu, Ni, Co, Zn and Mo are required but become toxic at very high concentration (Bayramoglu *et al*., 2003). This was further explained by Chadrasekaran and Rajkannan, (2003) and Nilanjana *et al*., (2008) that excess metal concentration interferes with fungal metabolic activities. In general, living cells are subjected to toxic effect of heavy metals, which perhaps, could result into cell death. Perhaps, the observed reduction in mycelial biomass at day 20 and 25 could be as a result of long exposure to the metals in the medium, which bioaccumulation, interfered with mycelial cell function, became toxic and retarded the mycelial growth (Nilanjana *et al*., 2008)

However, based on the correlation analysis using Pearson coefficient method, it was revealed that incubating period was inversely correlated with the levels of Ca, Zn, Cu, Co, and Cd in the filtrate but positively with Na while the relationship with Mg, K, P and Pb was not significant (Table 3). Therefore, in the filtrate, as the incubating period increased, the level of Na increased but those of Mg, Ca, Zn, Cu, Co and Cd decreased (the actual trend is shown in Fig. 2b). This means that at increasing incubating period there was a depletion in the levels of Ca, Zn, Co, Cu and Cd available for further cell functions in mycelia growth, which led into reduced biomass of mycelia after day 10 (0.90 mg/g with no significant weight at day 20 (0.15mg/g) and day 25 (0.010mg/g). Therefore, the observed reduction of mycelia growth after a peak at 10 days could be explained by the depletion of the minerals in the filtrate rather than the toxicity that could be from high concentration of metals in the filtrate. Correlation analysis showed that Na was inversely correlated with the rest of minerals; K was correlated with Ca, Pd, Co and Cd but not with Mg, P, Zn, and Cu; while the rest of the minerals were highly significant with one another (Table 3).

CONCLUSION

The fungus *L. squarrosulus* was able to utilize Na, K, Ca, Mg, Zn, P, Pb, Cu, Co and Cd in the brewery waste water for its metabolic life processes, thereby reducing their concentration levels and any further contamination/toxicity that might have resulted from the discharge of the wastewater into water bodies or its application on farm lands. Therefore, *L. squarrosulus* could be use as a bioremediation agent for brewery wastewater. In addition, the brewery wastewater could not support the growth of *L. squarrosulus* at day 20 and 25 due to the depletion of the minerals in the filtrate with increasing incubating period, indicating that the concentration of the metals in the waste is not high enough to cause metal toxicity and might partly be due to possible pretreatment of the wastewater.

Table 3: The correlation among incubating period and the assessed minerals among effluent, *L. squarrosulus*, cultured filtrate, and harvested mycelia

	Na	K	Mg	Ca	P	Pb	Zn	Cu	Co	Cd
Na	1.00	-0.62***	-0.31*	-0.60***	-0.31*	-0.57***	-0.44**	-0.50***	-0.59***	-0.70***
K		1.00	ns	0.42**	ns	0.33**	ns	ns	0.38**	0.49***
Mg			1.00	0.80***	0.84***	0.86***	0.90***	0.85***	0.75***	0.77***
Ca				1.00	0.70***	0.92***	0.88***	0.90***	0.93***	0.95***
P					1.00	0.81***	0.83***	0.79***	0.68***	0.71***
Pb						1.00	0.93***	0.91***	0.87***	0.95***
Zn							1.00	0.93***	0.85***	0.87***
Cu								1.00	0.93***	0.88***
Co									1.00	0.92***
	Correlation of incubating period (day) with minerals in the filtrate									
Day	0.50**	ns	-0.35*	-0.78***	ns	ns	-0.5**	-0.81***	-0.83***	-0.66***

* *Significant at p<0.5*
** *significant at p< 0.01*
*** *significant at p< 0.001*
ns *not significant*

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